Circ J 2007; **71:** 597 – 603

Improving Effects of Epigallocatechin-3-Gallate on Hemorheological Abnormalities of Aging Guinea Pigs

Huey-Chuan Cheng, MD; Chi-Ming Chan, MD*; Hsin-Sheng Tsay, PhD**; Hong-Jen Liang, PhD†; Yu-Chih Liang, PhD††; Der-Zen Liu, PhD‡

Background Epigallocatechin-3-gallate (EGCG) is the most potent antioxidant of all the green tea catechins. The objective of the present study was to find out whether it improved the age-induced hemorheological abnormalities or not.

Methods and Results Twenty-four-month-old aging guinea pigs were used to test the effects of EGCG on hemorheolohgical properties. Orally feeding EGCG at $30\,mg\cdot kg^{-1}\cdot day^{-1}$ for 28 days resulted in a decrease in erythrocyte membrane malondialdehyde, and further improved erythrocyte deformability and blood viscosity at high and middle shear rates. In addition, it also significantly reduced erythrocyte aggregation, and improved blood viscosity at low shear rates and viscoelasticity at oscillatory flow. Consequently, efficiency of blood oxygen transport in aged guinea pigs increased after administration with EGCG.

Conclusions Orally feeding EGCG $30\,mg\cdot kg^{-1}\cdot day^{-1}$ for 28 days significantly improves the abnormal hemorheological parameters. These results suggest that EGCG has considerable potential as a substantial component for the development of new drugs or functional foods in improving the age-induced hemorheological abnormalities. (*Circ J* 2007; **71:** 597 – 603)

Key Words: Aging; Blood viscosity; Epigallocatechin-3-gallate; Erythrocyte deformability; Malondialdehyde

Hemorheology is the study of properties of blood flow through the blood vessels in relation to biophysical parameters such as blood viscosity, erythrocyte deformability, erythrocyte aggregation and platelet aggregation. During the past 3 decades, strong correlations have been established between hemorheological abnormalities and cardiovascular diseases[1–4] cerebrovascular accident[5–7] and hypertension[8,9] Studies have indicated that hemorheological abnormalities increase the pathology of nervous system disorders such as Alzheimer's disease[10,11] and glaucoma[12–14]

Aging is an inevitable process undergone by all living creatures and it has been shown to be the main cause of hemorheological abnormalities. Many studies, using both animal and human models, have reported aging-induced hemorheological changes, such as increased fibrinogen concentrations[15,16] blood viscosity[17,18] plasma viscosity[19] and impaired erythrocyte deformability[20,21] Furthermore, it was believed that these abnormal hemorheological parameters might be etiologically related to the cumulative impacts of oxidative stress[22]

Our previous studies have showed the possible mechanism of the anti-tumor effect of epigallocatechin-3-gallate (EGCG). This effect is associated with EGCG's ability to block epidermal growth factor (EGF)[23] and inhibit EGF-induced cell transformation independently of AP-1 activation[24] By investigating the antioxidant activity through oxygen-radical absorbance capacity assay, regarding the tea extracts and tea polyphenols, EGCG has been shown to be the most potent radical scavenger[25] Although these results show that EGCG indeed has considerable anti-tumor and antioxidation activities that can potentially prevent microvascular disorders caused by oxidative stress, studies for the relationship between EGCG and microvascular disorders in terms of hemorheological abnormalities is relatively scarce. The present study aimed to assess the effects of EGCG on improvement of the hemorheological abnormalities of aging guinea pigs with the feeding treatment of EGCG at $10\,mg\cdot kg^{-1}\cdot day^{-1}$, $20\,mg\cdot kg^{-1}\cdot day^{-1}$ or $30\,mg\cdot kg^{-1}\cdot day^{-1}$, for 28 days, respectively. Hemorheological measurements such as blood viscosity at high, medium and low shear rates, and blood viscoelasticity under oscillatory flow were carried out. In addition, we also analyzed the erythrocyte deformability, lipid peroxidation of erythrocyte membrane, erythrocyte internal viscosity, erythrocyte aggregability and oxygen transport efficiency in attempt to obtain any correlations among them. Furthermore, the mechanism of EGCG in improvement of hemorheological abnormalities is discussed.

(Received October 6, 2006; revised manuscript received December 14, 2006; accepted January 12, 2007)

*Department of Ophthalmology, Mackay Memorial Hospital and Mackay Medicine, Nursing and Management College, *Department of Ophthalmology, Cardinal Tien Hospital, Taipei, **Institute of Biotechnology, Chaoyang University of Technology, Taichung, †Department of Food Science, Yuanpei University, Hsinchu, ††School of Medical Laboratory Science and Biotechnology, Taipei Medical University and ‡Graduate Institute of Biomedical Materials and Engineering, Taipei Medical University, Taipei, Taiwan*

Mailing address: Der-Zen Liu, PhD, 250 Wu-Hsing Street, Graduate Institute of Biomedical Materials and Engineering, Taipei Medical University, Taipei, Taiwan. E-mail: tonyliu@tmu.edu.tw

Table 1 Comparison of the Biochemical Parameters of Blood in 4-Month-Old Guinea Pigs (n=12) and 24-Month-Old Aging Guinea Pigs (n=12)

Parameters	*4-months-old guinea pigs mean±SD (n=12)*	*24-months-old ageing guinea pigs mean±SD (n=12)*	*Paired t-test p value*
MCV (fl)	*80.6±0.98*	*81.6±1.63*	*NS*
Hemoglobin (g/dl)	*15.2±1.41*	*14.4±1.83*	*NS*
Hematocrit (%)	*45.2±3.6*	*43.6±3.5*	*NS*
RBC (10^{12}/dl)	*5.6±0.44*	*5.3±0.46*	*NS*
Fibrinogen (mg/dl)	*258±55*	*339±31*	*<0.05*
Cholesterol (mg/dl)	*45±14.3*	*52±11.7*	*NS*

MCV, mean corpuscular cell volume of erythrocytes; RBC, red blood cell or erythrocytes.

Methods

Animals

Twelve and 48 guinea pigs at age 4 months and 24 months, respectively, were used in the current study. After 1 week of acclimation, the 24-month-old guinea pigs were randomized and divided into 4 groups. Control group (12 guinea pigs aged 24-month-old) and 12 guinea pigs aged 4 months received a normal diet and sterile water; 3 experimental groups A, B and C (12 guinea pigs aged 24 months, respectively for each group) were given equal amounts of a normal diet and water with different additions of EGCG (Sigma, 95%) dried powder (group A: $10\,mg\cdot kg^{-1}\cdot day^{-1}$; group B: $20\,mg\cdot kg^{-1}\cdot day^{-1}$; group C: $30\,mg\cdot kg^{-1}\cdot day^{-1}$), respectively. Duration of the oral administration of EGCG suspension was 28 days. At the end of the experiment, all animals were killed and blood samples from hearts were collected for the subsequent study. These animal experimental protocols were approved by Animal Ethics Committee of the Taipei Medical University.

Hemorheological Measurements

Plasma and Blood Viscosity Plasma and blood viscosity were measured using a Rheostress 1 double cone viscometer (HAAKE Mess-Technik, Karlsruhe, Germany), with a cone angle of 1° at 310 K. The serial blood viscosities at different shear rates were determined via a computer-controlled testing program. Shear rates of 500/s, 250/s, and 5/s, reflecting high, medium, and low shear rates were given. The viscoelasticity of the blood was tested in an oscillatory mode. The oscillatory shear strain was set at a constant 5% at frequencies ranging from 0.3 to 0.1 Hz.[26]

Erythrocyte Membrane Malondialdehyde (MDA) Analysis To measure the oxidative stress of erythrocyte membranes, the level of MDA, a product of lipid peroxidation which reacts with thiobarbituric acid (TBA), was examined by estimating the quantity of MDA-TBA complex at 532 nm with a spectrophotometer (Hitachi U2000, Hitachi, Japan).[27] The detailed preparation procedure for measuring the MDA-TBA complex was as an earlier report.[28] Quantities of MDA presented in the results are based on 10^{10} erythrocytes.[28]

Flow Resistance of Erythrocytes To measure the flow resistance of erythrocytes, we used constant flow rate filtration methods.[29] After separation from plasma by centrifuging the sample at 1,500 G for 10 min, erythrocytes were washed 3 times with phosphate-buffered saline. Then, erythrocyte suspensions at 5% hematocrit, were filtered through a 5-μm pore size. Nuclepore® membrane at a constant flow rate of 1.5 ml/min.The pressure-time data were measured with a pressure transducer connected to a Validyne digital transducer indicator. The continuous output data of the indicator were digitized and recorded on a computer. Recorded data were played back off-line, and Po values for ringer solutions and Pi values for erythrocyte suspensions were determined as previously reported.[30] Beta values were calculated with the data of Pi/Po and were indexed as the flow resistance of erythrocytes when flowing through the pores.[31]

The Deformability and Aggregability of Erythrocytes Erythrocytes deformability and aggregability were measured by laser diffractometer. The measurement of erythrocyte deformability was based on a laser diffraction method in which the laser beam traversed the diluted blood suspension (0.8 ml) and was diffracted by erythrocytes. The diffraction pattern was projected on a screen monitored by the photoelectric sensors, linked to a frame grabber integrated in the computer. The best fitting ellipse represented the deformed erythrocyte. The long and short axes, A and B, respectively, of this ellipse were used to calculate the deformation index: DI=(A–B)/(A+B). Before the measurement, the erythrocyte suspension was suspended in phosphate-buffered saline solutions with 5.5% polyvinylpyrrolidone. Details of the method have been described elsewhere.[30] For the erythrocyte aggregation test, 0.8 ml EDTA-blood sample was poured into the plate for which the diffraction of the laser radiation on the undiluted blood samples had been used for the aggregation degree estimation. The signal obtained from the intensity of the back-scattered light measured by the photoelectric sensors was further processed by the computer to estimate the aggregation degree. In our model, the optional disaggregation shear rate and its duration before stopping the motor were generally set at 600/s and 15/s, respectively. With syllectogram analysis for measuring erythrocyte aggregation, we obtain an aggregation index (AI) of erythrocytes.[32]

Erythrocyte Rigidity and Oxygen Transport Efficiency of the Whole Blood The index of eythrocyte rigidity (T_K) was calculated according to the equation of Dintenfass,[33] expressed as Equation 1. To avoid the influence of erythrocyte aggregation, the shear rate used in the Dintenfass equation should be greater than 180/s. Therefore, a shear rate of 250/s and 500/s were chosen in our experimental system. In addition, oxygen transport efficiency (T_E) of the blood is expressed as Equation 2 at a fixed shear rate.[34]

$$T_K=[(\eta^{0.4}-1)\eta^{0.4}]/Hct \quad (1)$$

$$T_E=Hct/\eta \quad (2)$$

Statistical Analysis

Statistical analysis of data were calculated with the SAS package (version 8.1, SAS Institute, Cary, NC, USA) and was performed using a one-way analysis of variance followed by Duncan's post-hoc test to compare the experimental groups and control group. A value of $p<0.05$ was selected as indicating statistical significance. Variability was expressed as the mean±standard deviation. Linear

Table 2 Comparison of Hemorheological Parameters in 4-Month-Old Guinea Pigs (n=12) and 24-Month-Old Aging Guinea Pigs (n=12)

Parameters	*4-months-old guinea pigs mean±SD (n=12)*	*24-months-old ageing guinea pigs mean±SD (n=12)*	*Paired t-test p value*
η *plasma (cp)*	*1.34±0.12*	*1.36±0.13*	*NS*
η *blood (cp)*^{S} (γ=*500 s*$^{-1}$)	*2.12±0.15*	*2.65±0.28*	*<0.01*
η *blood (cp)*^{S} (γ= *250 s*$^{-1}$)	*3.45±0.41*	*4.19±0.31*	*<0.01*
η *blood (cp)*^{S} (γ= *5 s*$^{-1}$)	*8.8±1.84*	*14.5±2.04*	*<0.01*
η' *blood (cp)*^{O}	*7.94±1.22*	*11.96±1.85*	*<0.01*
η" *blood (cp)*^{O}	*1.18±0.33*	*1.55±0.28*	*<0.05*

η plasma, the plasma viscosity of steadily flow; η blood, the whole blood viscosity of steadily flow; S, the steadily flow model of whole blood; γ, shear rate of steadily flow model (s); η' blood, the whole blood viscosity (oscillatory flow model); O, the oscillatory flow model of whole blood (0.1 Hz); η" blood, the whole blood elasticity (oscillatory flow model).

Table 3 Comparison of the Biochemical Parameters of Blood in 24-Month-Old Aging Guinea Pigs of Control Group (No Fed in EGCG) and Experimental Groups Fed With Different Quantities of EGCG

Parameters	*Control group mean±SD (n=12)*	*A group mean±SD (n=12)*	*B group mean±SD (n=12)*	*C group mean±SD (n=12)*
MCV (fl)	*81.6±1.63*^{a}	*81.9±1.73*^{a}	*81.4±1.88*^{a}	*81.3±1.85*^{a}
Hemoglobin (g/dl)	*14.4±1.83*^{a}	*14.5±1.93*^{a}	*14.6±2.06*^{a}	*14.6±1.93*^{a}
Hematocrit (%)	*43.6±3.5*^{a}	*43.9±3.8*^{a}	*44.4±3.9*^{a}	*44.5±4.2*^{a}
RBC (10^{12}*/dl)*	*5.3±0.46*^{a}	*5.4±0.48*^{a}	*5.4±0.52*^{a}	*5.5±0.56*^{a}
Fibrinogen (mg/dl)	*339±31*^{a}	*344±43*^{a}	*323±50*^{a}	*318±57*^{a}
Cholesterol (mg/dl)	*52±11.7*^{a}	*57±16.6*^{a}	*49±12.6*^{a}	*55±13.6*^{a}

A group: 10 mg·kg^{-1}*·day*$^{-1}$*; B group: 20 mg·kg*$^{-1}$*·day*$^{-1}$*; C group: 30 mg·kg*$^{-1}$*·day*$^{-1}$*.*
EGCG, epigallocatechin-3-gallate. Other abbreviations see in Table 1.

Table 4 Comparison of Hemorheological Parameters in 24-Month-Old Aging Guinea Pigs of the Control Group (No Fed in EGCG) and Experimental Groups Fed With Different Quantities of EGCG

Parameters	*Control group mean±SD (n=12)*	*A group mean±SD (n=12)*	*B group mean±SD (n=12)*	*C group mean±SD (n=12)*
η *plasma (cp)*	*1.36±0.13*^{a}	*1.35±0.15*^{a}	*1.34±0.19*^{a}	*1.26±0.08*^{b,*}
η *blood*^{S} *(cp)* (γ= *500 s*$^{-1}$)	*2.65±0.28*^{a}	*2.58±0.34*^{a,b}	*2.36±0.26*^{b,c,*}	*2.28±0.26*^{c,**}
η *blood*^{S} *(cp)* (γ= *250 s*$^{-1}$)	*4.19±0.31*^{a}	*4.12±0.32*^{a}	*4.06±0.42*^{a,b}	*3.75±0.33*^{b,**}
η *blood*^{S} *(cp)* (γ=*5 s*$^{-1}$)	*14.5±2.04*^{a}	*13.3±2.59*^{a}	*12.4±2.45*^{a,b}	*11.1±1.32*^{b,**}
η' *blood*^{O} *(cp)*	*11.96±1.85*^{a}	*11.13±2.43*^{a}	*10.69±2.83*^{a,b}	*8.89±2.02*^{b,**}
η" *blood*^{O} *(cp)*	*1.55±0.28*^{a}	*1.44±0.49*^{a}	*1.39±0.42*^{a,b}	*1.19±0.17*^{b,**}
AI	*2.72±0.21*^{a}	*2.55±0.27*^{a}	*2.47±0.37*^{a,b}	*2.12±0.25*^{b,**}

A group: 10 mg·kg^{-1}*·day*$^{-1}$*; B group: 20 mg·kg*$^{-1}$*·day*$^{-1}$*; C group: 30 mg·kg*$^{-1}$*·day*$^{-1}$*.*
AI, aggregate index of erythrocyte. Other abbreviations see in Tables 2,3.
**Represents p<0.05; **represents p<0.01.*

regressions higher than a 95% confidence level were also calculated by SigmaStat® Statistical Software (Jandel Scientific, San Rafael, CA, USA).

Results

Table 1 shows a comparison of blood biochemical parameters for guinea pigs aged between 4 months old and 24 months old. Except for fibrinogen concentration, no significant difference was found in erythrocyte counts, hematocrit and hemoglobin between the 2 groups. In addition, the cholesterol concentrations of the 24-month-old group were higher than that of the 4-mouth-old group, although there was no statistical difference between them. The comparison of hemorheological parameters listed in Table 2 showed that whole blood viscosity and blood viscoelasticity of the 24-month-old group were higher than those of the 4-month-old group but not for plasma viscosity.

Table 3 shows the values of blood biochemical parameters for aging guinea pigs after the oral treatment of EGCG. The results show that the aging guinea pigs in the experimental groups (A, B, C) fed with different quantities of EGCG did not show any significant change in erythrocyte counts, hemoglobin, mean corpuscular cell volume of erythrocytes (MCV), hematocrit concentrations, cholesterol concentrations and fibrinogen concentrations compared with the control group (no fed in EGCG). The hemorheological parameters shown in Table 4 showed that a significant reduction in plasma viscosity was found in group C, as well as the group's blood viscosity under the steady flow was significantly reduced at high (shear rate of steadily flow=500/s), medium (shear rate of steadily flow=250/s) and low (shear rate of steadily flow=5/s) shear rates as compared with the control group. The results of oscillatory flow showed significantly decreasing blood viscosity and viscoelasticity only in group C. Compared with the control group, erythrocyte aggregability (AI) of group C was also significantly decreased.

As the results of erythrocyte properties, aging guinea pigs in the experimental groups (A, B, C) fed in different

Table 5 Comparison of Hemorheological Parameters (Including Erythrocyte Rigidity, Deformaibility Index of Erythrocyte, Flow Resistance of Erythrocytes, MDA of Erythrocyte Membranes) in 24-Month-Old Aging Guinea Pigs of Control Group (No Fed in EGCG) and Experimental Groups Fed With Different Quantities of EGCG

Parameters	*Control group mean±SD (n=12)*	*A group mean±SD (n=12)*	*B group mean±SD (n=12)*	*C group mean±SD (n=12)*
T_K (γ= 500 s^{-1})	0.55±0.18[a]	0.55±0.17[a]	0.54±0.21[a]	0.53±0.21[a]
T_K (γ= 250 s^{-1})	0.85±0.21[a]	0.84±0.13[a]	0.82±0.24[a]	0.83±0.18[a]
MDA	5.91±0.40[a]	5.61±0.44[a,b]	5.33±0.45[b,c,*]	4.71±0.56[c,**]
DI (γ= 500 s^{-1})	0.31±0.02[a]	0.31 ±0.02[a,b]	0.35±0.04[b,c,*]	0.39±0.03[c,**]
DI (γ= 250 s^{-1})	0.26±0.04[a]	0.26 ±0.03[a]	0.29±0.04[a]	0.32±0.02[b,*]
β	10.11±0.88[a]	9.76 ±1.28[a]	9.28±0.99[a,b]	8.28±0.87[b,**]

A group: 10 mg·kg^{-1}·day^{-1}; B group: 20 mg·kg^{-1}·day^{-1}; C group: 30 mg·kg^{-1}·day^{-1}.
MDA, malondialdehyde of erythrocyte membrane; T_K, erythrocyte rigidity; DI, deformaibility index of erythrocyte; β, flow resistance of erythrocytes; γ, shear rate (s^{-1}). Other abbreviations see in Tables 2,3.
**Represents p<0.05; **represents p<0.01.*

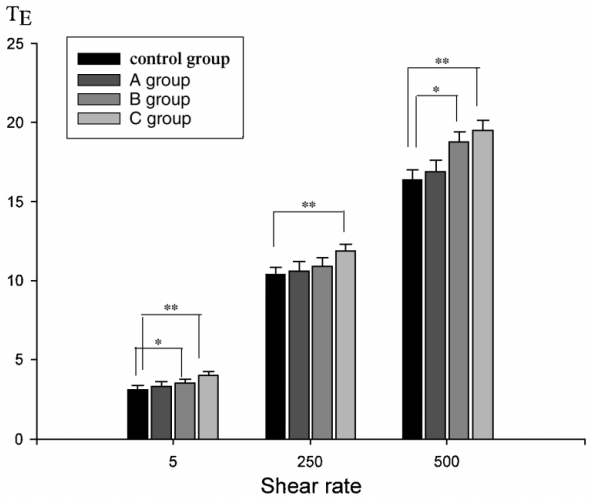

Fig 1. Comparison of the oxygen transport efficiency of whole blood measured as a function of different shear rates in 24-month-old aging guinea pigs of control group (no fed in epigallocatechin-3-gallate (EGCG)) and experimental groups (group A: 10 mg·kg^{-1}·day^{-1}; group B: 20 mg·kg^{-1}·day^{-1}; group C: 30 mg·kg^{-1}·day^{-1}) fed with different quantities of EGCG. In the figure, each column from left to right represents control group and experimental groups A, B and C groups, respectively. *represents p<0.05; **represents p<0.01. TE, oxygen transport efficiency.

quantities of EGCG did not show significant change in erythrocyte rigidity as compared with the control group (Table 5). However, lipid peroxidation (MDA) of erythrocyte membrane exhibited a significant decrease except for group A. Similarly, as expected, the erythrocyte deformability of groups B and C increased significantly at high and medium shear rates, meanwhile the flow resistance of erythrocytes was significantly decreased in the steady flow in groups B and C (Table 5). Furthermore, in groups B and C, the oxygen transport efficiency of whole blood was significantly increased at high, medium and low shear rates compared with the control group (Fig 1).

Figs 2a and b showed the linear correlation between the erythrocyte MDA and erythrocyte deformability, and between erythrocyte flow resistance index (β) and erythrocyte deformability for the control group, respectively. These results indicate that, before oral treatment of EGCG, increasing erythrocyte MDA concentrations might be correlated with decreasing erythrocyte deformability ($p<0.05$), and in turn decreasing erythrocyte deformability gives a rise in erythrocyte flow resistance in the control group ($p<0.05$). After the treatment with EGCG 30 mg·kg^{-1}·day^{-1} for 28 days (group C) as shown in Figs 3a and b, good negative linear correlations were produced between erythrocyte MDA concentrations and erythrocyte deformability ($p<0.05$), and erythrocyte deformability and erythrocyte flow resistance index ($p<0.05$). Therefore, oral intake of EGCG over a period of time significantly decreased erythrocyte MDA concentrations and further improved erythrocyte deformability impairments.

Discussion

In general, the aging process is believed to produce changes in the vascular system and in hemorheological parameters, for example, a rise in blood viscosity and plasma viscosity. These cumulative effects of hemorheological abnormalities appears in the form of a disturbed blood flow profile in older individuals, leading to the development or aggravation of various circulatory disorders, such as cardiovascular disease, cerebrovascular accident and hypertension. The results in our animal study show that EGCG might significantly improve the age-induced hemorheological abnormalities. For improving the health and quality of life of older adults, oral administration of EGCG might have a vast potential as a therapeutic agent for the prevention of the age-induced abnormal hemorheological behaviors.

One of the often seen consequences of the aging process is the alterations in blood biochemical parameters. For example, Yamarat et al reported that adults had higher fibrinogen concentration and blood viscosity and lowered MCV than newborns.[35] Coppola et al further indicated that the elder person not only has higher fibrinogen concentrations but also has lower erythrocyte counts![18] However, except for fibrinogen cocentrations, the biochemical parameters of blood in the present study showed no significant difference between the 24-month-old group and the 4-month-old group (Table 1). In addition, the results also show that oral intake of EGCG over a period of 28 days did not cause any significant changes in blood biochemical parameters (Table 3) in 24-months-old aging guinea pigs.

The correlation between hemorheological properties and the aging process has been extensively investigated by numerous studies. Erythrocyte deformability in 150-, 320- and 710-day-old rats studied by Abe et al has shown that impaired erythrocyte deformability of ageing rats could result in increased blood viscosity.[36] Kameneva et al showed that aging led to a decrease in erythrocyte deformability

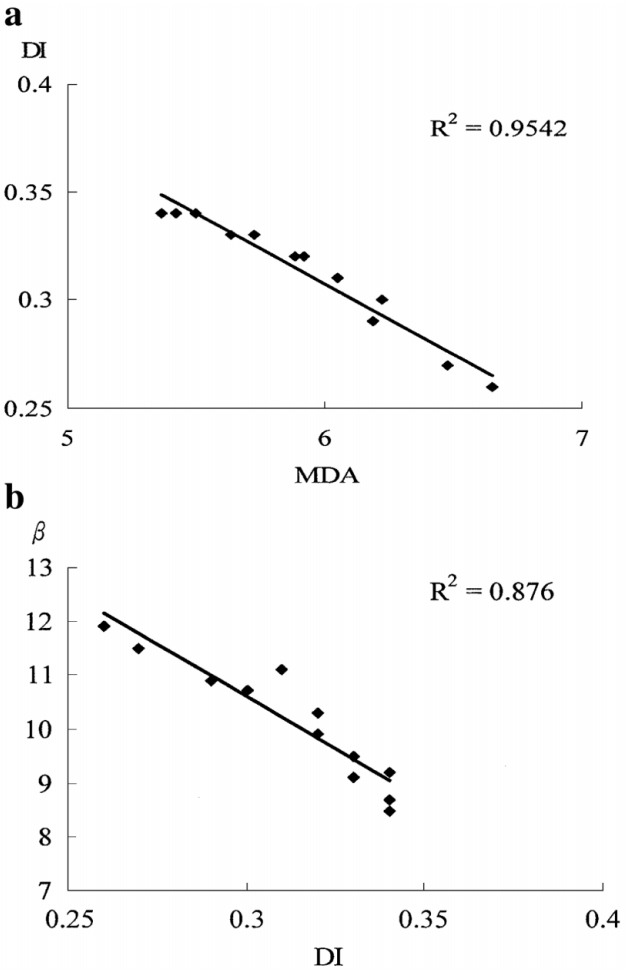

Fig 2. (a) Linear correlations between the erythrocyte malondialdehyde (MDA) and deformability of erythrocyte (DI) in 24-month-old aging guinea pigs (control group). (b) Linear correlation between the erythrocyte flow resistance index (β) and DI in 24-month-old aging guinea pigs (control group).

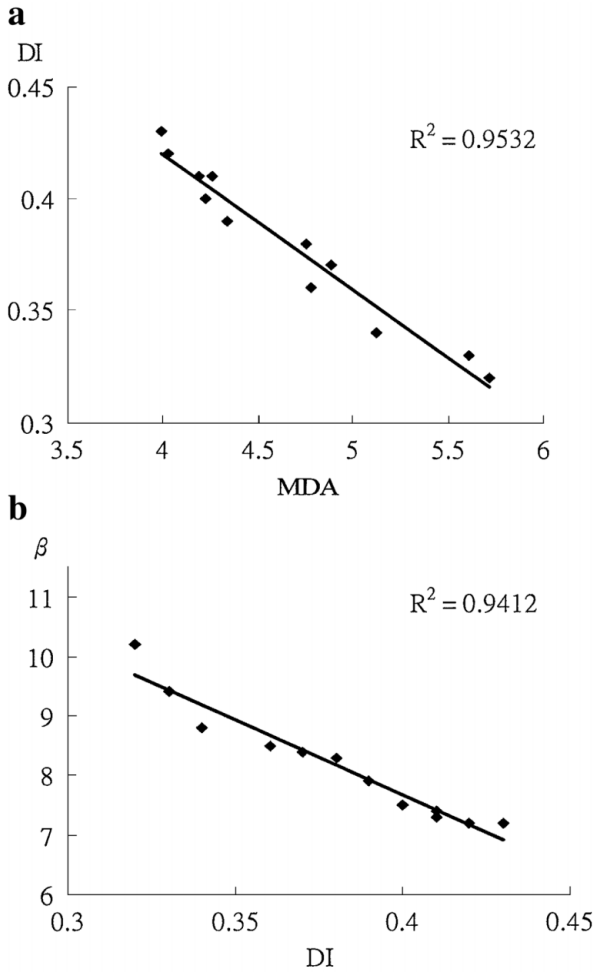

Fig 3. (a) Linear correlations between the erythrocyte malondialdehyde (MDA) and deformability of erythrocyte (DI) in 24-month-old aging guinea pigs (group C). (b) Linear correlation between the erythrocyte flow resistance index (β) and DI in 24-month-old aging guinea pigs (group C).

and an increase in both erythrocyte aggregation and blood viscosity.[37] Ajmani and Rifkind showed that the aging process raised erythrocyte rigidity, plasma viscosity and blood viscosity.[38] Oder et al showed that erythrocyte aggregation at a low shear rate was increased by aging.[39] These findings were in good agreement with our results (Table 2). In the present study, significant difference in hemorheological parameters was found between the 24-month-old group and the 4-month-old group. It is feasible to use 24-month-old aging guinea pigs as an appropriate animal model to screen and test new drugs and functional foods for improving the age-induced hemorheological abnormalities.

In the present study, we used 2 different flow fields of hemodynamics to simulate blood flow and measure the blood viscosity and viscoelasticity. In steady flow, our results show that oral feeding of EGCG for 28 days clearly improved blood viscosity of aging guinea pigs in group C (Table 2). It is generally known that decreased blood viscosity at high shear rate was dominated by an increase in erythrocyte deformability, although decreased blood viscosity at a low shear rate is dependent on reduced erythrocyte aggregation. Moreover, the model of oscillatory flow, shear stress was dominated by frequency-dependent sinusoidal flow instead of continuous flow. In our results, after being fed EGCG for 28 days, group C showed a significant reduction in blood viscosity and elasticity (Table 4). We believe that the main cause for this is probably reduction in the aggregation of erythrocytes.

Erythrocyte deformability has been thought to have a detrimental influence on peripheral microcirculation or the blood's oxygen transport efficiency. The previous investigation of Kameneva et al and Terranova et al showed that the aging process significantly decreases erythrocyte deformability.[37,40] Tillmann et al reported that the aging-induced impaired erythrocyte deformability that accompanies aging was a result of an increase in erythrocyte rigidity.[41] This suggests that erythrocyte deformability is greatly dependent on mechanical properties of the membrane and internal viscosity (erythrocyte rigidity) of erythrocytes. However, in our present study, no significant change was found in the erythrocyte rigidity of group A, B or C compared with the control group, but the significant decrease in the MDA concentrations of the erythrocyte membrane is quite interesting. MDA is known to be an end product of lipid peroxidation and is mainly formed under oxidative stress. The decrease in MDA concentrations of aging guinea pigs caused by oral

feeding of EGCG was, we assume, a result of EGCG acting as an antioxidant by trapping free radicals and inhibiting lipid peroxidation.

Interestingly, we found (as shown in Fig 2 and Fig 3) negative correlations between erythrocyte membrane MDA and erythrocyte deformability, and between erythrocyte deformability and the flow resistance of erythrocytes. This means that either before or after oral administration of EGCG, lipid peroxidation in erythrocyte membrane leads to decreased erythrocyte deformability. Subsequently, as erythrocyte deformability was impaired, the flow resistance of erythrocytes also inevitably increased. Even more important is the finding that a high oral intake of EGCG apparently improved the state of lipid peroxidation in the erythrocyte membrane, which consequently improved erythrocyte deformability and decreased flow resistance of erythrocytes (Fig 3).

High erythrocyte aggregability not only increases the blood viscosity under low shear rate but it is also a risky factor for the formation of blood clots. The mechanism of erythrocyte aggregation is an extremely complicated process and depends on the interaction (attractive) force between fibrinogen and erythrocytes, which is correlated with erythrocyte surface charge distribution, surface geometry shape and fibrinogen concentrations. Despite the fact that oral intake of EGCG did not cause significant reduction in fibrinogen concentrations, our data show a clear reduction in erythrocyte aggregability. This reduction is probably as a result of a more flexible erythrocyte membrane (good deformability) and decreasing interactions in erythrocytes and fibrinogens, which further decreased erythrocyte aggregation.

The decreasing blood viscosity or blood viscoelasticity can be attributed to several possible factors, including decreased plasma viscosity, decreased erythrocyte aggregation, or increased erythrocyte deformability. The present study suggests that the oral intake of EGCG at 30 mg/day for 28 days seems sufficient to induce a reduction in the erythrocyte membrane MDA of aging guinea pigs with a subsequent improvement in erythrocyte deformability. This improvement results in decreased resistance of erythrocyte and blood viscosity at high shear rate. In addition, our results also show that a high oral intake of EGCG reduces erythrocyte aggregation, leading to a decrease in blood viscosity at a low shear rate, and an increase in the oxygen-carrying capability of whole blood.

In conclusion, EGCG given orally to 24-month-old aging guinea pigs for 28 days caused no significant difference in blood biochemical parameters and fibrinogen concentrations, but did improved abnormal hemorheological parameters. EGCG reduced the MDA of erythrocyte membrane and further increased erythrocyte deformability and improved the flow resistance of erythrocytes. Also, the increased erythrocyte deformability might subsequently decrease the blood viscosity at high and middle shear rates. In addition, the oral intake of EGCG significantly reduced erythrocyte aggregation, improving blood viscosity at low shear rates and viscoelasticity at oscillatory flow. Furthermore, the oxygen transport efficiency of blood in aging guinea pigs also increased after 28 days of EGCG oral intake.

Acknowledgments

We thank the Mackay Memorial Hospital, Taipei, Taiwan and the Department of Health, Executive Yuan, Taiwan for financially supporting this work under Grant No. 93MMH-TMU-12 and No. DOH-94-TD-F-113-028, respectively.

References

1. Lipowsky HH. Microvascular rheology and hemodynamics. *Microcirculation* 2005; **12:** 5–15.
2. Lowe GD, Rumley A, Whincup PH, Danesh J. Hemostatic and rheological variables and risk of cardiovascular disease. *Semin Vasc Med* 2002; **2:** 429–439.
3. Ratnayake WM, L'Abbe MR, Mueller R, Hayward S, Plouffe L, Hollywood R, et al. Vegetable oils high in phytosterols make erythrocytes less deformable and shorten the life span of stroke-prone spontaneously hypertensive rats. *J Nutr* 2000; **130:** 1166–1178.
4. Steiner S, Jax T, Evers S, Hennersdorf M, Schwalen A, Strauer BE. Altered blood rheology in obstructive sleep apnea as a mediator of cardiovascular risk. *Cardiology* 2005; **104:** 92–96.
5. Szapary L, Horvath B, Marton Z, Alexy T, Kesmarky G, Habon T, et al. Short-term effect of low-dose atorvastatin on haemorrheological parameters, platelet aggregation and endothelial function in patients with cerebrovascular disease and hyperlipidaemia. *CNS Drugs* 2004; **18:** 165–172.
6. Grasso G. An overview of new pharmacological treatments for cerebrovascular dysfunction after experimental subarachnoid hemorrhage. *Brain Res Brain Res Rev* 2004; **44:** 49–63.
7. Banerjee R, Nageswari K, Puniyani RR. Association of hemorheological parameters and risk of stroke in hypertensives of Indian origin. *Clin Exp Hypertens* 2000; **22:** 687–694.
8. Lip GY, Blann AD, Farooqi IS, Zarifis J, Sagar G, Beevers DG. Abnormal haemorheology, endothelial function and thrombogenesis in relation to hypertension in acute (ictus <12 h) stroke patients: The West Birmingham Stroke Project. *Blood Coagul Fibrinolysis* 2001; **12:** 307–315.
9. Sandhagen B. Red cell fluidity in hypertension. *Clin Hemorheol Microcirc* 1999; **21:** 179–181.
10. Wen Z, Xie J, Guan Z, Sun D, Yao W, Chen K, et al. A study of hemorheological behaviour for patients with Alzheimer's disease at the early stages. *Clin Hemorheol Microcirc* 2000; **22:** 261–266.
11. Solerte SB, Ceresini G, Ferrari E, Fioravanti M. Hemorheological changes and overproduction of cytokines from immune cells in mild to moderate dementia of the Alzheimer's type: Adverse effects on cerebromicrovascular system. *Neurobiol Aging* 2000; **21:** 271–281.
12. Hamard P, Hamard H, Dufaux J. Hemo-rheology of glaucomatous neuropathy. *Bull Soc Belge Ophtalmol* 1992; **244:** 17–25 (in French).
13. Egorov VV, Bachaldin IL, Sorokin EL. Impact of disorders in blood rheology on progress of glaucoma in patients with stable normalized intraocular pressure. *Vestn Oftalmol* 1999; **115:** 5–7.
14. Ates H, Uretmen O, Temiz A, Andac K. Erythrocyte deformability in high-tension and normal tension glaucoma. *Int Ophthalmol* 1998; **22:** 7–12.
15. Papet I, Dardevet D, Sornet C, Bechereau F, Prugnaud J, Pouyet C, et al. Acute phase protein levels and thymus, spleen and plasma protein synthesis rates differ in adult and old rats. *J Nutr* 2003; **133:** 215–219.
16. Schuitemaker GE, Dinant GJ, van der Pol GA, van Wersch JW. Fibrinogen levels in hypercholesterolemic smokers and non-smokers in relation to age and gender. *Clin Exp Med* 2004; **3:** 231–235.
17. Ajmani RS, Metter EJ, Jaykumar R, Ingram DK, Spangler EL, Abugo OO, et al. Hemodynamic changes during aging associated with cerebral blood flow and impaired cognitive function. *Neurobiol Aging* 2000; **21:** 257–269.
18. Coppola L, Caserta F, De LD, Guastafierro S, Grassia A, Coppola A, et al. Blood viscosity and aging. *Arch Gerontol Geriatr* 2000; **31:** 35–42.
19. Konstantinova E, Tolstaya T, Prishchep S, Milutin A, Mironova E, Ivanova L. Plasma lipid levels, blood rheology, platelet aggregation, microcirculation state and oxygen transfer to tissues in young and middle-aged healthy people. *Clin Hemorheol Microcirc* 2004; **30:** 443–448.
20. Kameneva MV, Garrett KO, Watach MJ, Borovetz HS. Red blood cell aging and risk of cardiovascular diseases. *Clin Hemorheol Microcirc* 1998; **18:** 67–74.
21. Muravyov AV, Draygin SV, Eremin NN, Muravyov AA. The microrheological behavior of young and old red blood cells in athletes. *Clin Hemorheol Microcirc* 2002; **26:** 183–188.
22. Rifkind JM, Ajmani RS, Heim J. Impaired hemorheology in the aged associated with oxidative stress. *Adv Exp Med Biol* 1997; **428:** 7–13.
23. Liang YC, Lin-shiau SY, Chen CF, Lin JK. Suppression of extracellular signals and cell proliferation through EGF receptor binding

by (–)-epigallocatechin gallate in human A431 epidermoid carcinoma cells. *J Cell Biochem* 1997; **67:** 55–65.
24. Dong Z, Ma W, Huang C, Yang CS. Inhibition of tumor promoter-induced activator protein 1 activation and cell transformation by tea polyphenols, (–)-epigallocatechin gallate, and theaflavins. *Cancer Res* 1997; **57:** 4414–4419.
25. Lin YL, Juan IM, Chen YL, Liang YC, Lin JK. Composition of polyphenols in fresh tea leaves and associations of their oxygen-radical-absorbing capacity with antiproliferative actions in fibroblast cells. *J Agric Food Chem* 1996; **44:** 1387–1394.
26. Liu DZ, Chien SC, Tseng LP, Yang CB. The influence of hyperbaric oxygen on hemorheological parameters in diabetic rats. *Biorheology* 2003; **40:** 605–612.
27. Stocks J, Dormandy TL. The autoxidation of human red cell lipids induced by hydrogen peroxide. *Br J Haematol* 1971; **20:** 95–111.
28. Jain SK, McVie R, Duett J, Herbst JJ. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. *Diabetes* 1989; **38:** 1539–1543.
29. Huang SY, Jeng C, Kao SC, Yu JJ, Liu DZ. Improved haemorrheological properties by Ginkgo biloba extract (Egb 761) in type 2 diabetes mellitus complicated with retinopathy. *Clin Nutr* 2004; **23:** 615–621.
30. Schonbein HS, Ruef P, Linderkamp O. The shear stress diffractometer rheodyn SSD for determination of erythrocyte deformability: Principle of operation and reproducibility. *Clin Hemorheol Microcirc* 1996; **16:** 745–748.
31. Schmalzer EA, Skalak R, Usami S, Vayo M, Chien S. Influence of red cell concentration on filtration of blood cell suspensions. *Biorheology* 1983; **20:** 29–40.
32. Hardeman MR, Dobbe JG, Ince C. The laser-assisted optical rotational cell analyzer (LORCA) as red blood cell aggregometer. *Clin Hemorheol Microcirc* 2001; **25:** 1–11.
33. Dintenfass L. Internal viscosity of the red cell: Problems associated with definition of plasma viscosity and effective volume of red cells in the blood viscosity equation. *Biorheology* 1975; **12:** 253–256.
34. Messmer K, Schmid-Schmid S-H, Chien S, Karger H. Present state of blood rheology in hemodilution. *Theor Basis Clin Appl* 1972; **1:** 1–45.
35. Yamarat P, Sanghirun C, Chantachum Y, Cheeramakara C, Thanomsak W. Factors influencing blood viscosity: Adult and newborn blood analysis. *Southeast Asian J Trop Med Public Health* 2000; **31**(Suppl 1)**:** 75–78.
36. Abe H, Orita M, Arichi S. Erythrocyte deformability in aging. *Mech Ageing Dev* 1984; **27:** 383–390.
37. Kameneva MV, Garrett KO, Watach MJ, Borovetz HS. Red blood cell aging and risk of cardiovascular diseases. *Clin Hemorheol Microcirc* 1998; **18:** 67–74.
38. Ajmani RS, Rifkind JM. Hemorheological changes during human aging. *Gerontology* 1998; **44:** 111–120.
39. Oder W, Kollegger H, Baumgartner C, Zeiler K, Oder B, Deecke L. Age and whole blood viscoelasticity. A risk factor study. *Acta Med Austriaca* 1991; **18**(Suppl 1)**:** 71–74 (in German).
40. Terranova R, Alberghina M, Carnazzo G. Lipid composition and fluidity of the erythrocyte membrane in the elderly. *Ric Clin Lab* 1985; **15**(Suppl 1)**:** 327–334 (in Italy).
41. Tillmann W, Levin C, Prindull G, Schroter W. Rheological properties of young and aged human erythrocytes. *Klin Wochenschr* 1980; **58:** 569–574.