

Anti-Lipid Peroxidative Effects of Ling Zhi in Mice

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Abstract The inhibitory effect of Ling Zhi (LZ) and Vit E on the malondialdehyde (MDA) content produced by Fe^{2+} -Vit C system in rat liver mitochondria in vitro were studied. The experiment showed that LZ (4.0 mg/ml) and Vit E (1 mmol/L) could completely inhibit the level of MDA induced by Fe^{2+} -Vit C system. Ten-days successive administration of LZ (0.75, 1.5, 3.0 g/kg) ip. could result in significant inhibition of lipid-peroxidation induced by 50% alcohol on heart, liver and hepatic mitochondria of mice and also decrease CCl_4 -induced lipid-peroxidation products-MDA in the serum of mice. Besides, LZ inhibited the lipid-peroxidation on sera of normal mice. The results indicated that LZ might cause a protective effect on the mouse heart and liver damaged by alcohol and CCl_4 .

Key words Ling Zhi (LZ); Lipid-peroxidation; Malondialdehyde (MDA).

Studies have showed that active oxygens and free radicals generated in the biological systems are related to toxicity, disease and aging^(1~2). Active oxygens and free radicals might induce lipid peroxidation in biological cell membranes, which subsequently leads to the production of a series of compounds including conjugated dienes, malondialdehyde (MDA), fluorescent substances, etc. It has also suggested that lipofuscin, an age-pigment, accumulates in the brain, heart and skin tissues through lipid peroxidation. Therefore, protection of membranes from abnormal lipid-peroxidation is essential for human health and to delay the aging processes.

LZ is a traditional tonic and has the effect of prolonging life and preventing diseases, such as hypertension, hypercholesterol and liver damage, etc. Previous report from our department implicated that LZ could scavenge superoxide anion generated by Xanthine-Xanthine Oxidase system (see 16 chapter of this book). In this paper, we study the anti-lipid peroxidative effects of LZ, using Fe^{2+} -Vit C system in vitro and 50% alcohol, CCl_4 intoxicated mice.

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Materials and Methods

Animals

Mice Kunming (KM), half male and half female, 20 ± 2 g; SD rats, male, 250 ± 50 g were supplied by the Animal Center, Shanghai Medical university.

Reagents

LZ was provided by Wakan Shoyaku Botany Institute, Tokyo, Japan.

LZ (0.25 g/ml) was incubated in a shaking water bath at 80°C for 4 h. After cooling, the suspension was frozen at -20°C for 1 h then thawed at 37°C water bath. The steps were repeated three times. After centrifuge 30 000 g 15 min, the supernatant was used in the experiment. 2-Thiobarbituric Acid (TBA) and 1,1,3,3 – Tetraethoxypropane were purchased from Sigma Chemical Co. Phosphotungstic acid, Carbon tetrachloride (CCl_4), Ethyl alcohol absolute and Butyl alcohol (BuOH) were purchased from Shanghai Chemical Plant.

Equipments

721-Spectrophotometer, HITACHI 650-60 Fluorescence, RC5-C centrifuge.

Preparation of rat hepatic mitochondria and experiment ⁽⁴⁾

The rats were decapitated and the liver was quickly removed and placed in ice-cold buffer (Tris 0.1 mol/L, KCl 1 mol/L, pH 7.4), homogenized at 10% (W/V) for 30s. The tissue homogenate was centrifuged 600g at 4°C 15 min. The supernatant was centrifuged 10 000 g at 4°C 15 min and the pellet resuspended in Tris-buffer (mitochondria). Protein concentration was determined by Coommas Bright Blue method.

The hepatic mitochondria suspension 0.5 ml (containing 1 mg protein) were incubated in a water bath at 37°C for 20, 40 and 60 min respectively, in the presence of 0.005 mmol/L FeSO_4 , 0.1 mmol/L Vit C with or without LZ (0.5 to 4.0 mg/ml), Vit E as a control drug. The final reaction volume was 1 ml. Following incubation, the reaction tubes were added to 2 ml 0.1 mol/L HCl, 1 ml 0.67% TBA, heated for 15 min in a boiling water bath and extracted with 4 ml BuOH after cooling, shaking 3 ~ 5 min. The BuOH phase was separated by centrifuged (3 000 rpm, 10 min) and the optical density (O.D.) was measured at 535 nm with 721 spectrophotometer.

Experiment of mice in vivo

1. Five groups of mice (10 mice each) were administered LZ suspended in normal saline (NS), ip. at doses of 0 (two groups), 0.75 (one group), 1.5 (one group) and 3.0 (one group) g/kg/d, respectively. Ten successive days while the animals were given 50% Alcohol, po. at a dose of 12 ml/kg/d to four groups. One control group was given NS only. The animals were fasted 8 h after the last administration and killed by decapitation. The heart and liver were quickly taken and homogenized, then 0.5 ml heart or liver homogenate, or hepatic mitochondria mentioned above was added 2 ml 0.1 mol/L HCl, 1 ml 1% TBA and 0.5 ml Tris-buffer. The mixture was heated for 15 min, 4 ml BuOH added, shaking 3 ~ 5 min, centrifuged 3 000 rpm 10 min. Extract with BuOH was measured by the O.D. value for MDA determined.

2. Six groups of mice (10 mice each) were given LZ suspended in NS, po. LZ at doses of 0.188, 0.75 and 3.0 g/kg/d (each dose for two groups) for ten successive days. Other three groups were given NS for control. After the last administration, the animals were fasted 8 h, then intoxicated with a mixture of CCl₄ and vegetable oil (1:1, V/V, 60µl/20g body weight), ip. in half LZ groups and one NS group. One NS group was given vegetable oil only. 2 h after CCl₄-intoxication, all mice were killed and the blood was quickly sampled. The other half LZ groups and one NS group were also killed and sampled, the blood taken for a non-toxication experiment. The blood was stored at 4°C for 24 h. The serum was taken from blood centrifuged at 2 000 rpm for 15 min.

The determination of MDA in sera⁽⁵⁾

30 µl serum, 2 ml 1/24 mol/L H₂SO₄ and 0.5 ml 10% Phosphotungstic acid were shaken up, kept 5 min and centrifuged (3 000 rpm) for 10 min. The pellet was resuspended in 2 ml 1/24 mol/L H₂SO₄, 0.3 ml 10% Phosphotungstic acid and centrifuged (3 000 rpm) for 10 min. The pellet was again resuspended in 1 ml water, 1 ml 0.67% TBA-acetic acid (1:1, V/V) and shaken up for 3 ~ 5 min. The mixture was heated for 1 h in a boiling water bath and extracted with 3.5 ml BuOH after cooling. The BuOH phase was separated by centrifuged (3 000 rpm 10 min) and the fluorescent substances were determined by HITACHI 650-60 Fluorescence (excitation at 515 nm and emission at 553 nm).

Data analysis

Based on the following equation the fluorescent substances were calculated as following:

$$\text{MDA nmol/ml} = 0.5 \times \frac{f - B}{F - B} \times \frac{1.0}{0.03} = 16.7 \frac{f - B}{F - B}$$

f is the sample tube, B is a blank tube and F is a standard tube. The calibration substances were prepared with 1, 1, 3, 3 – Tetraethoxypropane. The data were shown as $\bar{x} \pm SD$, and statistical significance was evaluated by the t test.

Results

The effect of LZ on Fe^{2+} -Vit C system in vitro

As shown in Fig. 17-1 and Tab. 17-1, Fe^{2+} -Vit C system resulted in a significant increase of the relative levels of MDA, compared with control group. After LZ added (0.5 ~ 4.0 mg/ml), the relative levels of MDA were significantly decreased. At a dose of 4.0 mg/ml. The decrease rate was 100%. Compared with normal mitochondria, the relative amount of MDA was no significant difference. Vit E had the same effect as LZ. MDA decreased 90% when Vit E was less than 10 mmol/L (Fig. 17-2, Tab. 17-2).

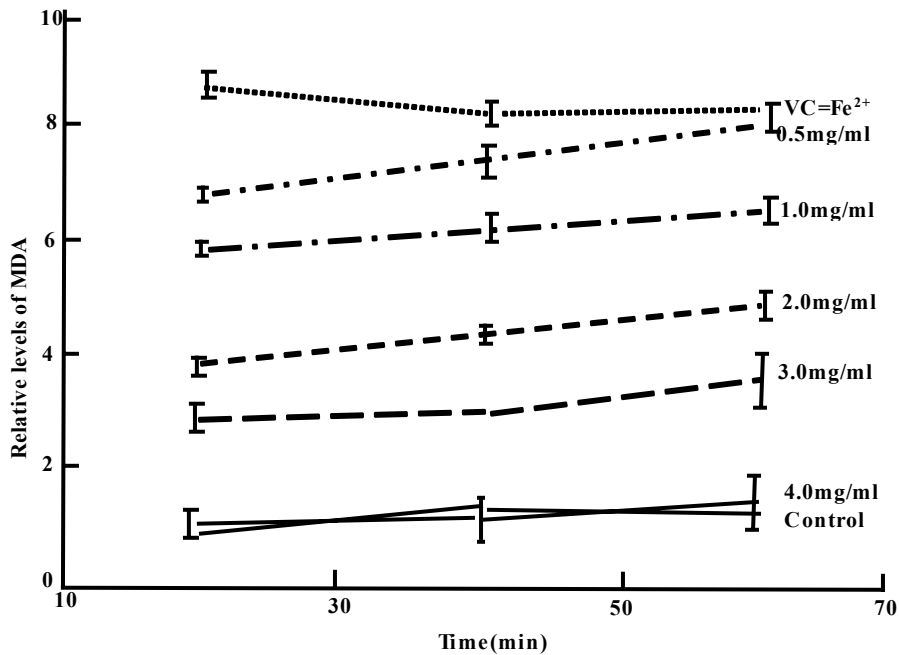


Fig. 17-1 The anti-lipid peroxidation effect of LZ in mouse liver mitochondria in vitro

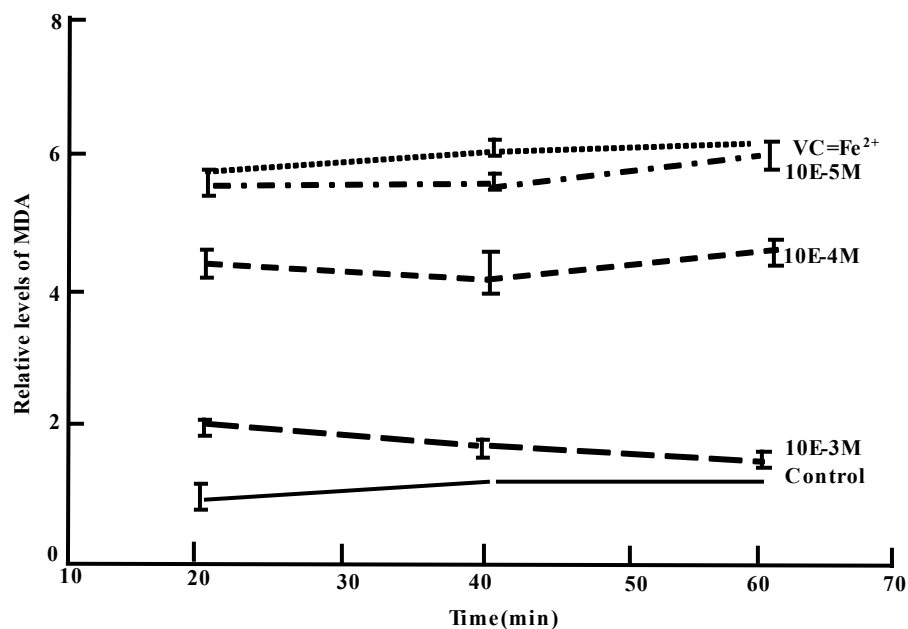


Fig. 17-2 The anti-lipid peroxidation effect of Vit E in mouse liver mitochondria in vitro

Tab. 17-1 Effect of LZ on the relative levels of MDA in rat mitochondria in vitro*

Time (min)	Control (n=4)	Fe ²⁺ +Vit C (n=4)	LZ (n = 4, mg/ml)				
			0.5	1.0	2.0	3.0	4.0
20	1.0 ± 0.2	8.8 ± 0.6	6.9 ± 0.1	5.9 ± 0.1	3.8 ± 0.1	3.8 ± 0.3	1.1 ± 0.2
40	1.5 ± 0.2	8.4 ± 0.1	7.6 ± 0.3	6.5 ± 0.3	4.5 ± 0.2	3.2 ± 0.1	1.3 ± 0.4
60	1.6 ± 0.3	8.8 ± 0.2	8.5 ± 0.1	6.9 ± 0.2	5.2 ± 0.3	3.9 ± 0.5	1.8 ± 1.5

*Compared with those of Fe²⁺ +Vit C group, the relative levels of MDA in all groups treated with LZ were significantly decreased, P < 0.01

Tab. 17-2 Effect of Vit E on the relative levels of MDA in rat mitochondria in vitro*

Time (min)	Control (n=4)	Fe ²⁺ +Vit C (n=4)	Vit E (n=4, mol/L)		
			10 ⁻⁵	10 ⁻⁴	10 ⁻³
20	1.0 ± 0.2	5.8 ± 0.1	5.5 ± 0.2	4.4 ± 0.2	1.9 ± 0.2
40	1.4 ± 0.1	6.2 ± 0.1	5.8 ± 0.1	4.3 ± 0.4	1.8 ± 0.1
60	1.5 ± 0.1	6.4 ± 0.1	5.3 ± 0.3	4.8 ± 0.2	1.8 ± 0.1

*Compared with those of Fe²⁺ +Vit C group, the relative levels of MDA in all groups treated with Vit E were significantly decreased, P < 0.01

The effect of LZ on mice in vivo

The lipid peroxidation caused by alcohol in heart, liver and the hepatic mitochondria of mice, as expressed by MDA content, were markedly increased (64%, 66%, 74%, respectively) (Tab. 17-3). However, administration of LZ (0.75, 1.5, 3.0 g/kg/d) led to a significant decrease of MDA levels of the heart, the liver and the hepatic mitochondria. At a dose of 0.75 g/kg, the level of MDA of hepatic mitochondria trended to decrease without significant difference.

Tab. 17-3 The inhibitory effect of LZ on 50% alcohol induced heart and liver peroxidation in mice

Treatment	N	Dose	Heart MDA	Liver MDA (nmol/g prot.)	
			(nmol/g prot.)	Homogenate	Mitochondrion
Control	9		200.71 ± 82.51	21.36 ± 6.09	37.95 ± 6.18
Alcohol	6	12 ml/kg	328.59 ± 51.71***	35.53 ± 8.70***	65.86 ± 13.75***
LZ	6	0.75 g/kg	220.58 ± 47.50***	24.86 ± 5.25**	57.07 ± 12.93
+ Alcohol		12 ml/kg			
LZ	8	1.50 g/kg	220.06 ± 38.56***	21.52 ± 7.48***	38.94 ± 16.42***
+ Alcohol		12 ml/kg			
LZ	10	3.00 g/kg	234.79 ± 37.33***	18.82 ± 5.10***	38.61 ± 11.26***
+Alcohol		12 ml/kg			

** P<0.05 and ***P<0.01, compared with alcohol group. *** P<0.01, compared with control group

Tab. 17-4 showed the effects of LZ on the levels of MDA in serum of normal mice (without CCl₄ intoxication). The results indicated that LZ could significantly decrease lipid peroxidation in serum of normal mice with dose-dependence. There was no significant effect observed at a dose of 0.188 g/kg.

Tab. 17-4 Effect of LZ on MDA in the serum of normal mice

Treatment	N	Dose (g/kg)	MDA in serum (nmol/ml)
Control	10		12.7 ± 1.7
LZ	8	0.188	12.1 ± 1.1*
LZ	8	0.750	10.7 ± 1.5***
LZ	9	3.000	9.2 ± 0.2***

*P > 0.05 and *** P < 0.01 compared with control group

Tab. 17-5 Effect of LZ on MDA in serum of mice intoxicated with CCl₄

Treatment	N	Dose (g/kg)	CCl ₄ Intoxication	MDA in Serum (nmol/ml)
Control	7	—	—	12.0 ± 1.4
CCl ₄ treated	8		+	14.7 ± 1.5***
LZ + CCl ₄	7	0.188	+	12.8 ± 3.9*
LZ + CCl ₄	8	0.750	+	10.5 ± 0.9***
LZ + CCl ₄	10	3.000	+	9.9 ± 1.4***

* P > 0.05 and *** P < 0.01 compared with CCl₄ treated group

*** P < 0.01 compared with control group. +: CCl₄ intoxication; —: without CCl₄ intoxication

By intoxication 2 h with CCl₄ to mice, the MDA levels in serum significantly increased with preadministration of LZ (0.75, 1.5, 3.0 g/kg qd. x 10) caused a significant decrease of MDA level (Tab. 17-5).

Discussion

LZ is a well known anti-aging medicinal herb. Some authors found that the acetone extract of LZ decreased lipid peroxidation in vitro ⁽⁶⁾. Using the Fe²⁺ -Vit C system producing lipid-peroxidation in rat hepatic mitochondria in vitro, we proved that the water extract of LZ significantly decreased the levels of MDA and supported the anti-lipid peroxidation action of LZ. Vit E, as a major antioxidant factor, could prevent membrane peroxidative damage. The same action of Vit E and LZ on MDA level might have a same biochemical basis. It may dependent on the action of glutathione peroxidase (GSH-Px) and decrease of fatty acid hydroperoxide formation ^(7, 8). In Tab. 17-2 and Fig. 17-2, the results showed that Vit E inhibited the lipid peroxidative products (inhibited rate 90%).

The animals administrated with CCl₄ or alcohol might result in the generations of free radicals such as CCl₃·, CClO· or C₂H₅O· in liver or other tissues, which led to lipid peroxidation, then cell death, tissue damage, even severe liver diseases. The levels of MDA formed through chain reactions induced by free radicals on the cell membrane reflected the level of the lipid peroxidation. This parameter could provide a convenient assay for lipid-peroxidation and cellular membrane damage. In this study, the results demonstrated that administration of LZ had the anti-lipid peroxidative action and protect the heart and the liver against injury induced by Alcohol or CCl₄. Meanwhile, the results showed that LZ also decreased the levels of MDA in non-toxication mice, indicating that there was the antilipid peroxidation effect of LZ to normal mice. As previous report ⁽³⁾,

LZ showed a direct inhibition in superoxide anion generated by Xanthine-Xanthine Oxidase system. This data suggested that scavenging cytotoxic oxygen free radicals may be one of the important pharmacological mechanisms for the anti-lipid peroxidative effect of LZ. Further work is needed to assess whether there are a ability of LZ to elevate SOD or GSH-Px activity or other antioxidant factors in animal tissues.

Effects of Ling Zhi on Membranes Fluidity and Ghosts Reseal Ability of Rat Erythrocyte

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Abstract Effects of Ling Zhi (LZ) on membranes fluidity and ghosts reseal ability of rat erythrocyte were studied by measuring the change in fluorescence polarization and the activity of NADH-K₃Fe(CN)₆ cytochrome C oxidoreductase with enzyme method respectively. The results showed that the fluorescence polarization, average microviscosity and anisotropy were significantly decreased after rats given LZ 125, 250, 500 mg/kg, ig. Qd. x 15 in comparison with NS control group. It suggests that LZ may increase the membrane fluidity. The reseal ability of erythrocyte membranes (ghost) were also significantly elevated after rats given LZ 250 or 500 mg/kg, ig. qd. x 15.

Key words Ling Zhi (LZ); Membrane fluidity of erythrocyte; Ghost reseal ability.

LZ is a famous and precious Chinese traditional medicine, which has been recommended by physicians and pharmacists. In many data it accumulated about pharmacological research work of LZ. In our department previous researches demonstrated that LZ possessed the marked protective actions for eliminating O⁻ free radical, raising the metamorphosis capacity of RBC, improving blood fluidity and decreasing blood viscosity^(1, 2). For clarifying the molecular mechanism of LZ actions mentioned above, we studied the effects of LZ on membranes fluidity and ghosts reseal of rat erythrocyte with positive control of Royal Jelly.

Materials and Methods

Animals

Wistar rats, ♀♂, 198.0 ± 7.8 g, supplied from the Animal Center, Shanghai.

Reagents

LZ (*Ganoderma Lucidum*) extract was provided by Wakan Shoyaku Botany Institute, Tokyo, Japan. The 5% suspension was prepared with 0.2% CMC solution, shaking in 80°C water bath 5 h. The stock suspension was then diluted according to final concentrations: 25.0 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.12 mg/ml, respectively.

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1,6-Diphenyl-1, 3, 5-hexatriene (DPH, lot No. 267592 1188) was purchased from Fluka Co. The 2 mM DPH prepared with tetrahydrofuran was stored in refrigerator and diluted to 2×10^{-6} M with 10 mM phosphate-buffered saline (PBS) before use.

Royal Jelly (RJ), Concentration 30 mg/ml was purchased from Shanghai First Chinese Medicine Works.

Equipments

MPF-4 fluorescence spectrophotometer; F-3000 fluorescence spectrophotometer; 721-spectrophotometer.

Preparation of erythrocyte membranes

Rats were randomly divided into 6 groups, 10 in each. The drugs were given to each group according to following dose, ig, qd. x 15, respectively. a) NS, b) RJ 150 mg/kg, c) LZ 500 mg/kg. d) LZ 250 mg/kg. e) LZ 125 mg/kg. f) LZ 62.5 mg/kg.

Rats blood was obtained by decapitation and anticoagulated with heparin. A single stage hemolysis method in hypotonic solution was used for the preparation of erythrocyte membranes⁽³⁾, in general term “ghost”. Blood was centrifuged at 2 000 rpm for 10 min. The supernatant was removed. The residual RBC was washed twice in NS for removing WBC, platelets and fibrinogen. The washed RBC was resuspended in cold 10mM Tris-HCl buffer pH 7.4 (v/v, 1:40), and mixed by gentle swirling. The erythrocyte ghost was isolated 1 h after standstill by centrifugation at 4 000 rpm for 10 min and diluted properly to a concentration of 200 ~ 800 μ g/ml. Protein contents of the samples were assayed with Lowry’s method⁽⁴⁾.

Measurement of erythrocyte membranes fluidity

1. Excitation and emission spectrogram of membranes labeled with DPH: 2 ml of 2 mM DPH was added to 2 ml of ghost preparation and incubated in water bath at 25°C for 30 min. After centrifugation at 4 000 rpm for 10 min the precipitate was collected and washed with 10 mM PBS. The membranes were resuspended in 4 ml of PBS. The excitation and emission spectrogram of membranes were scanned by F-3 000 fluorescence spectrophotometer.

2. Measurement of fluorescence polarization and average microviscosity of membranes: According to the reference⁽⁵⁾,. The fluorescence polarization (P) of rat erythrocyte membranes labeled with DPH for each group were determined by MPF-4 fluorescence spectrophotometer ($\lambda_{ex} = 362$ nm, $\lambda_{em} = 432$ nm). The degree of polarization was calculated as:

$$P = \frac{I_{VV} - GI_{VH}}{I_{VV} + GI_{VH}} \qquad G = \frac{I_{HV}}{I_{HH}}$$

Where I_{VV} and I_{VH} are the intensity of fluorescence, which are recorded at the two analyzing orientation, i.e. when the emission light polarized with electric light vector vertically or horizontally. G is a correction factor. Since the emission is passed through an analyzing monochromator which itself has a polarizing effect, G can be used to correct the relative transmission of the emission monochromator for the two polarization directions. According to Azumi's method⁽⁶⁾, I_{HH} is the intensity of emitted light when the two analyzing polarizer orientation are at horizontal directions. I_{HV} is the intensity of emitted light when the emission polarizer at horizontal and the excitative polarizer at vertical orientation. As compared with liquid, a measure of the microviscosity is used to show the fluidity of membranes. From the following formula, the average microviscosity ($\bar{\eta}$) or anisotropy (r) further calculated to represent the fluidity of membranes.

$$\bar{\eta} = \frac{2P}{0.46 - P} \qquad r = \frac{2P}{3 - P}$$

3. Ghosts reseal ability of erythrocytes:

① Preparation of sealed ghosts⁽⁷⁾: Blood was anticoagulated with heparin and washed twice in NS for removing WBC, platelets and fibrinogen. The washed red cells were suspended in cold 5mM PBS (containing $MgSO_4$ 1 mM), pH 8.0, PBS: RBC = 40:1, 1 h after mixture the erythrocyte ghost was isolated by centrifugation at 20 000 g for 40 min, and washed twice again in PBS to remove hemoglobin as far as possible to eliminate the influence in the determination.

② Determination of reseal ability: Membranes impermeability reflects that ghosts may be resealed in isotonic solution and returned the action of permeability barrier for large molecules and cations. After ghost reseal, NADH- $K_3Fe(CN)_6$ cytochrome C oxydoreductase (presumed it reside on inside of the membrane) cannot be measured. When saponin (0.1% in PBS) was used to disrupt the permeability barrier, the maximum activity of the enzyme was exhibited. The ability of the sealed ghosts was measured by Steck's method⁽⁷⁾. The activity of NADH-cytochrome C oxydoreductase was measured for same sample at two different conditions with or without saponin. Reseal ability of ghost (impermeability) was calculated as:

$$\text{Impermeability} = \frac{\text{Enzyme activity with saponin} - \text{Enzyme activity without saponin}}{\text{Enzyme activity with saponin}} \times 100\%$$

Results

Membranes fluidity of erythrocytes

1. Protein contents of membranes in each group

The protein contents of erythrocyte membranes measured by Lowry's method were shown in Tab. 18-1.

Tab. 18-1 Protein contents of the erythrocyte membranes

Group	n	Protein content ($\bar{X} \pm SD$ $\mu\text{g/ml}$)
NS	10	512.0 \pm 110.7
RJ (150 mg/kg)	10	502.1 \pm 105.9
RJ (500 mg/kg)	10	527.2 \pm 159.2
RJ (250 mg/kg)	10	523.9 \pm 119.9
RJ (125 mg/kg)	10	509.5 \pm 105.5
RJ (62.5 mg/kg)	10	546.4 \pm 115.8

The results showed that there were no significant differences between the treated groups ($P > 0.05$).

2. Excitation and emission spectrum of membranes labeled with DPH

The excitation and emission spectrum scanned by F-3 000 fluorescence spectrophotometer were shown in Fig. 18-1, 2.

The spectrum scanned showed that they had maximum excitation and emission peak at $\lambda_{\text{ex}} = 362$ nm, $\lambda_{\text{em}} = 432$ nm, respectively.

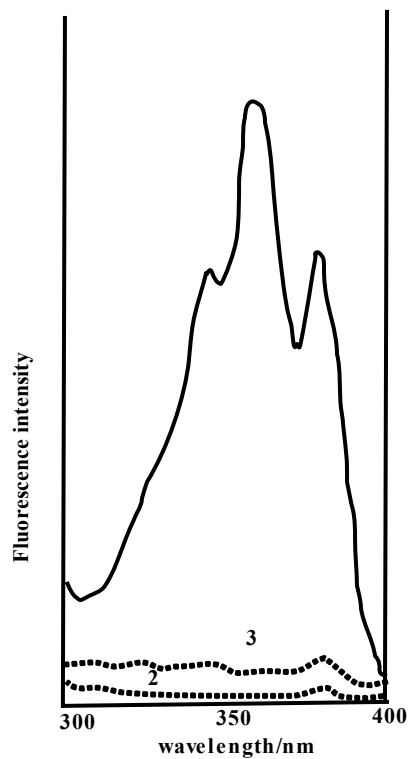


Fig. 18-1 Excitation spectrogram of erythrocyte membrane
1. Labelled with DPH
2. Unlabelled
3. DPH reagent.
slit: 5.5 nm, $\lambda_{em}=432$ nm

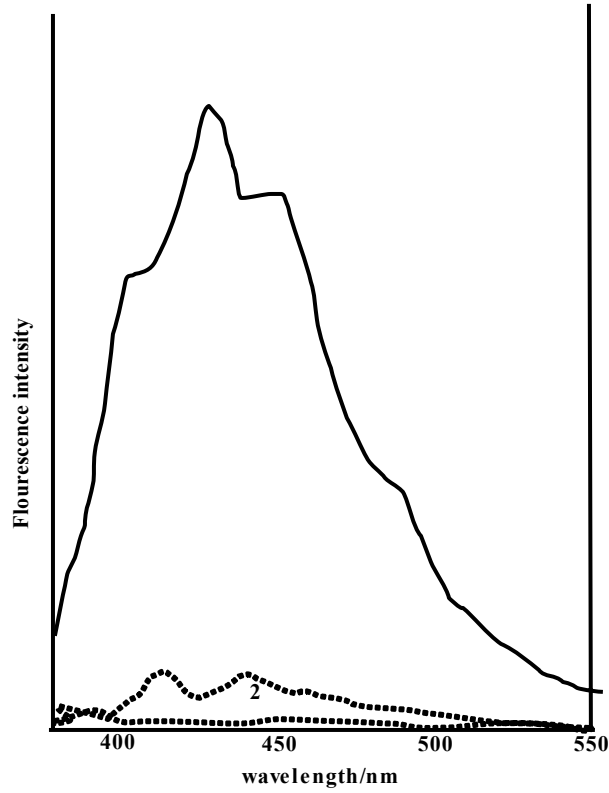


Fig. 18-2 Emission spectrogram of erythrocyte membrane
 1. Labelled with DPH
 2. Unlabelled
 3. DPH reagent.
 slit: 5.5 nm, $\lambda_{ex}=362\text{nm}$

3. The polarization (P), average microviscosity ($\bar{\eta}$) and anisotropy (r) of rat erythrocyte membranes labeled with DPH in each group were shown in Tab. 18-2.

Tab. 18-2 P, $\bar{\eta}$ and r of erythrocyte membranes

group	P	η ($\eta / \times 10^3$ protein)	R
NS	0.306 ± 0.020	4.07 ± 0.82 (80.2 \pm 9.11)	0.227 ± 0.016
RJ (150 mg/kg)	$0.289 \pm 0.013^*$	3.40 ± 0.42 (69.5 \pm 11.57)*	$0.213 \pm 0.011^*$
LZ (500 mg/kg)	$0.272 \pm 0.026^{**}$	2.97 ± 0.61 (58.8 \pm 12.98)**	$0.200 \pm 0.021^{**}$
LZ (250 mg/kg)	$0.282 \pm 0.022^*$	3.22 ± 0.58 (64.4 \pm 14.93)*	$0.207 \pm 0.018^*$
LZ (125 mg/kg)	$0.287 \pm 0.005^*$	3.32 ± 0.15 (67.8 \pm 13.99)*	$0.212 \pm 0.004^*$
LZ (62.5 mg/kg)	0.305 ± 0.014	3.97 ± 0.56 (74.4 \pm 11.37)	0.226 ± 0.011

$\bar{x} \pm \text{SD}$; n = 10; *P<0.05; **P<0.01 compared with NS

The polarization (P), microviscosity ($\bar{\eta}$) and anisotropy (r) are smaller, the lipid membrane fluidity is larger. The results showed that P, $\bar{\eta}$ and r values of rat erythrocyte membranes decreased significantly after RJ 150 mg/kg ig. qd. x 15. It means RJ increases membrane fluidity. It was consistent with that of reference⁽⁸⁾, P, $\bar{\eta}$ and r values decreased significantly after LZ 150, 250 and 500 mg/kg ig. qd. x 15. LZ elevates membrane fluidity of rat erythrocyte. However, there are no significant differences between LZ and RJ groups.

Reseal ability of erythrocyte ghosts

The results determined for the impermeability of erythrocyte membranes was shown in Tab, 18-3.

The results showed that impermeability of erythrocyte membrane elevated significantly after LZ 500 and 250 mg/kg ig. qd. x 15 (P < 0.05) and RJ 150 mg/kg ig. However, these was no significant difference between them.

Tab. 18-3 Reseal ability of erythrocyte ghosts. $\bar{x} \pm SD$, n = 10

Group	NADH-cytochrome C activity		Reseal ability (%)
	- saponin	+ saponin	
NS	0.0045 ± 0.0028	0.0045 ± 0.0028	53.1 ± 5.0
RJ (150 mg/kg)	0.0042 ± 0.0018	0.0042 ± 0.0018	59.8 ± 3.5*
LZ (500 mg/kg)	0.0040 ± 0.0029	0.0040 ± 0.0029	59.8 ± 3.5**
LZ (250 mg/kg)	0.0044 ± 0.0018	0.0044 ± 0.0018	58.9 ± 3.2*
LZ (125 mg/kg)	0.0044 ± 0.0013	0.0044 ± 0.0013	55.3 ± 4.6
LZ (62.5 mg/kg)	0.0045 ± 0.0017	0.0045 ± 0.0017	53.7 ± 5.0

$\bar{x} \pm SD$; n = 10; *P<0.05; **P<0.01 compared with NS

Discussion

The membrane fluidity of erythrocyte is a physical nature which indicates the flow of various membrane systems being similar to that of liquid state. The membrane fluidity can be effected by many factors, including the constituent of lipid membranes, environmental factors, the action of xenogenic substances, etc. The membrane fluidity has often been changeable during the process of membranes completing its function. Current studies showed that the membrane fluidity could be usually altered in the process of diseases. When the fluidity decreases, the membrane protein is more easily exposed in

water solution and have an effect on the activity of enzymes. At normal physiological condition, the body can regulate automatically to suit these changes. When the body cannot regulate automatically, it will be sick. The membrane fluidity of erythrocyte decrease in aging with saturate fatty acid contents increasing. Elevating membrane fluidity may possess the action of antisenility. It is reported that membranes reseal ability of erythrocyte is relevant to the age of cells. The membrane of erythrocyte will lose the reseal ability with cells aging. For example, membranes reseal ability of young erythrocyte is about 92.9%, however, that of old age erythrocyte is only 35.7%. From this view, drugs, which elevate membranes reseal ability of erythrocyte, may possess the certain actions of antisenility. In addition, since membranes of young erythrocyte itself possess more potent reseal ability than that of old cells, LZ elevating membranes reseal ability of aging erythrocyte retards senility.

Effects of Ling Zhi on Superoxide Dismutase Activity and Protein Components of Rat Erythrocyte Membranes

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Abstract Effects of Ling Zhi (LZ) on superoxide dismutase (SOD) activity and protein components (high molecular polymers HMP) of erythrocyte membranes were studied after rats given 62.5, 125, 250 and 500 mg/kg, ig. qd. x 15 respectively. By comparison with NS control group, results showed that SOD activity was significantly increased ($P < 0.05$) after 125, 250 and 500 mg/kg, ig. qd. x 15. There were no effect of LZ on protein components in all groups ($P > 0.05$).

Key words Ling Zhi (LZ); Superoxide dismutase (SOD); High molecular polymer (HMP).

LZ is a precious Chinese traditional medicine. It was considered to possess actions of nourishing, tonic and prolong live. It has been demonstrated in our department that LZ possesses the pharmacological actions of eliminating O free radical, immunoregulation⁽¹⁾, increasing RBC metamorphosis ability⁽²⁾ erythrocyte membrane fluidity and ghost reseal ability. This paper is to study the effects of LZ on superoxide dismutase (SOD) activity and high molecular polymers (HMP).

Materials and Methods

Animals

Wistar rat, ♀ ♂, 198.0 ± 7.8 g, supplied from the Animal Center, Shanghai.

Reagents

Ling Zhi (LZ *Ganoderma Lucidum*, Fr. Karst) extract was provided by Wakan Shoyaku Botany Institute, Tokyo, Japan. The suspension of 5% LZ was prepared with 0.2% CMC solution, shaking in 80°C water bath 5 h. The stock suspension was diluted to final concentrations: 25.0, 12.5, 6.25, 3.12 mg/ml, respectively.

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Royal Jelly (RJ, 30 mg/ml), was purchased from Shanghai First Chinese Medicine Works. It was diluted to 7.5 mg/ml.

Superoxide dismutase (SOD) standard was obtained from Shanghai Institute of Biochemistry, Chinese Academy of Sciences, activity 100 000 U/mg enzyme.

Equipments

Hitachi low temperature high velocity centrifugator; Beckman TLC Scanner; 721-spectrophotometer.

Preparation of erythrocyte membranes

Rats were randomly divided into 6 groups, 10 in each. According to following dose, drugs were given to each group, ig. qd. x 15, respectively. a) NS. b) RJ 150 mg/kg. c) LZ 500 mg/kg. d) LZ 250 mg./kg. e) LZ 125 mg/kg. f) LZ 62.5 mg/kg.

Rats blood was obtained by decapitation and anticoagulated with heparin. A single stage hemolysis method in hypotonic solution was used for the preparation of erythrocyte membranes⁽³⁾. Blood was centrifuged at 2 000 rpm for 10 min. The supernatant was removed. The residual RBC was washed twice with NS for removing WBC, platelets and fibrinogen. The washed RBC was suspended in cold 10 mM Tris-HCl buffer, pH 7.4 (v/v, 1:40), and mixed by gentle swirling for 1 ~ 2 min. The erythrocyte membranes was isolated 1 h, after standstill by centrifugation at 4 000 rpm for 10 min and washed twice with NS again. Protein contents of the samples were assayed with Lowry's method⁽⁴⁾.

Assay of SOD activity

0.5 ml washed erythrocyte membrane was measured by pyrogallol-NBT colorimetry for SOD activity⁽⁵⁾. Protein contents of the samples were assayed with Lowry's method⁽⁴⁾.

Measurement of HMP of membranes

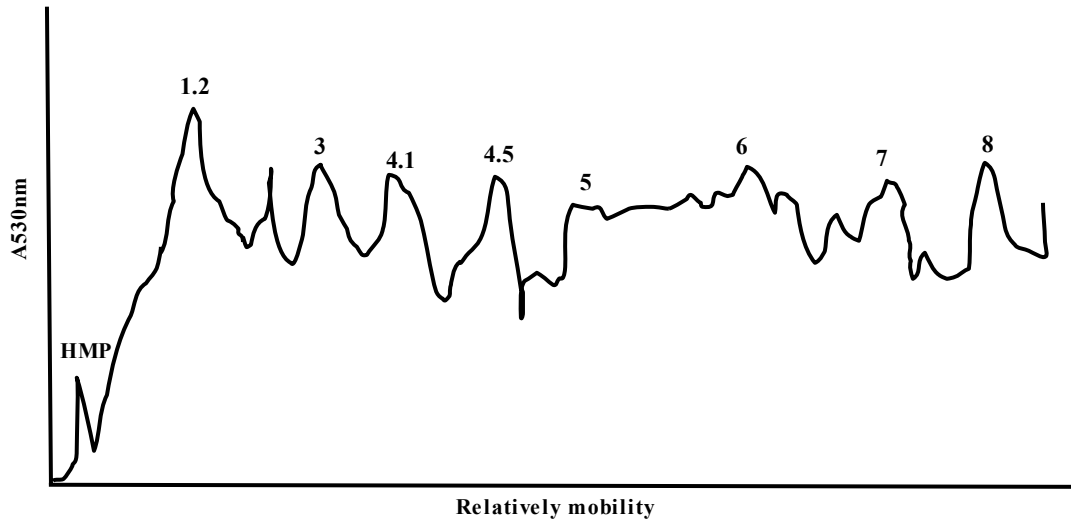


Fig. 19-1 The SDS-PAGE patten of erythrocyte membranes (X 530nm)

The membrane proteins could be crosslinked into polymers when erythrocyte membranes were damaged by oxidation. As a result the membrane permeability and the enzyme activity might be obstructed.

The HMP content may reflect the damaged degree of membranes. Membranes prepared above were treated with 1 mM pyrogallol solution, the components of membrane proteins (HMP) were measured with SDS-PAGE method⁽⁶⁾. Protein profile scanned by SDS-PAGE gel electrophoresis for rate erythrocyte membrane was shown in Fig. 19-1.

HMP band were just in front I and II bands. There was no HMP band of membrane untreated with pyrogallol.

Results

Activity of SOD in each group of rats

The SOD activity of erythrocyte membranes in each group was shown in Tab. 19-1.

Tab. 19-1 Effect of LZ on the activity of SOD of erythrocyte membranes

Group	SOD activity/mg protein
NS	36.6 ± 6.8
RJ (150 mg/kg)	46.5 ± 6.7*
LZ (500 mg/kg)	48.8 ± 4.9**
LZ (250 mg/kg)	45.8 ± 6.4*
LZ (125 mg/kg)	44.2 ± 7.4*
LZ (62.5 mg/kg)	41.2 ± 6.8

$\bar{x} \pm SD$; n = 10; *P<0.05; **P<0.01 compared with NS group

Results indicated that the SOD activity of membranes was significantly increased (P < 0.01 or 0.05) after LZ 500, 250 or 125 mg/kg, ig. qd. x 15 with dose-effectiveness. RJ exhibited obvious effect too (P < 0.05).

HMP contents of rat erythrocyte membranes

Tab. 19-2 Effect of LZ on HMP contents of erythrocyte membranes

Group	SOD activity/mg protein
NS	0.65 ± 0.48
RJ (150 mg/kg)	0.22 ± 0.17*
LZ (500 mg/kg)	0.31 ± 0.27
LZ (250 mg/kg)	0.36 ± 0.35
LZ (125 mg/kg)	0.57 ± 0.37
LZ (62.5 mg/kg)	0.56 ± 0.39

$\bar{x} \pm SD$; n = 10; *P<0.05 compared with NS group

The HMP contents of erythrocyte membranes in each group was shown in Tab. 19-2.

Results indicated that the HMP content was significantly decreased by RJ 150 mg/kg ig. qd. x 15 ((P < 0.05). There were no significant differences between LZ groups and control group.

Discussion

Superoxide anions (O_2^-) is a main free radical in biological body with the important significance in the study of aging recently. O_2^- is harmful to the body and can be one of the mechanisms inducing inflammation, aging, and cancer, etc. ⁽⁷⁾. Superoxide dismutase, being a new preparation of treatable enzyme, is an important enzyme of eliminating oxygenic free radical. SOD possesses protective action on human erythrocyte membrane⁽⁸⁾, which can elevate the fluidity and reseal ability of membranes, decrease crosslinked action of membrane protein. Previous studies in our department showed that LZ possesses the actions of eliminating O_2^- , elevating fluidity of lipid membranes. In this paper we are determining the effects of LZ on SOD activity directly. Results provides a reliable evidence of the protective action of LZ on erythrocyte membrane.

When cell membranes are damaged by oxidation the HMP contents may reflect the damaging degree of membrane due to the crosslinking of membranes proteins⁽⁹⁾. In this paper, results showed that LZ decreased the contents of HMP only at doses of 500 and 250 mg/kg respectively. However there are no significant differences observed. It may be related to many factors. This study was performed only on normal erythrocyte membrane. The further study will be sought.

The component of LZ is very complex and its quality is difficult to be controlled. Our experiment only provided some evidences for the application of LZ in the field of antisenility.

Effects of Ling Zhi on Experimental Thrombosis and Metamorphosis of Human Erythrocyte

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Abstract Following oral administration, Ling Zhi (LZ) extract was found to show the actions against the thrombosis of both platelet and fibrin in rats, and raising the metamorphosis capability of aging red cells in human body. But there were no effects on the prothrombin time of rats and partial thrombin of kaolin.

Key words Ling Zhi (LZ); Thrombosis; Metamorphosis capacity of erythrocyte.

The plant using in this paper was artificially cultured Ling Zhi (LZ, *Ganoderma Lucidum*, Fr. Karst). LZ extract was provided by Wakan Shoyaku Botany Institute, Tokyo, Japan. We studied the effects of it on experimental thrombosis in rats and metamorphosis capability of red blood cells (RBC) in human body.

Materials, Subjects and Methods

Animals

Wistar rat, ♀, supplied by the Animal Center, Shanghai Medical University.

Reagents

LZ dry extract is the powder in brown color provided by Wakan Shoyaku Botany Institute, Tokyo, Japan.

Preparation of 5% LZ Carboxyl Methyl Cellulose (CMC) suspension: 5g LZ dry extract was added to 0.5% CMC solution to obtain 5% LZ suspension. Oscillated for 4h in 80°C water bath and cooled in 4°C refrigerator for using.

Preparation of LZ in water solution:

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Water solution I : LZ CMC suspension was oscillated 2 h in 80°C water bath. The supernatant was used after centrifugation.

Water solution II: LZ CMC suspension was oscillated 2 h with ultrasonic wave (40w, 30s). The supernatant was used after centrifugation.

Sodium chloride injection (pH 7.2 ~ 7.4), was the Product of Chang Zheng Pharmaceutical Factory, Shanghai.

Plasm poor whole blood (fresh, any blood types) was provided from Central Station of Blood Transfusion, Shanghai.

Equipments

1. DXC-300A apparatus for metamorphosis capability of RBC, provided from Shanghai Medical University.
2. DS-87A model thrombus apparatus, provided from Develop Service Company of Science and Technology, Dantu, Jiangsu Province, China.

Platelet aggregation in rats in vivo

When the platelet in arterial blood flow of animal attached to the rough surface of silk thread and adhered to it, the aggregate substance of platelet rounded on the surface of thread to form platelet thrombus. Due to the action of adhesion-aggregation of platelet decreased, the thrombus weight decreased. According to the thrombus weight the action of adhesion-aggregation of platelet might be detected.

Rats with starvation 12 ~ 14 h before experiment were randomly divided into different groups. Drugs were given (po.) 2 times with interval of 1.5 h. 1.5 h after 2nd dose sodium pentobarbital 40 mg/kg was given (ip.) to anaesthetize.

A weighted silk thread, 5 cm in length, was putted into the middle position of a polyvinyl casing pipe with heparin, containing of 3 polyvinyl tubes, inserted into left external carotid vein and right common carotid artery respectively. The blood flow was opened and the extracorporeal circulation was formed. Circulation was stopped 15 min later, the silk thread were taken out and weighted quickly. Total weight minus weight of silk thread itself, it was the net weight of thrombus. The positive control group was using aspirin.

Rat experimental thrombosis (Chandlers method)

The siliconated polyvinyl tube is 60 mm in length and 4 mm in internal diameter. The two ends of the tube were brought together and joined by an outside plastic collar to form a circle being 38 mm in diameter. The circular tube was securely placed and centered on a turntable, tilted to an angle of 74°, and rotated at 18 rpm. Speed of blood

flow in circle is 419 mm/min. The above procedure could be carried out in 15 min. The laboratory temperature was $37 \pm 0.1^\circ\text{C}$. Platelet aggregation and prothrombin activation in circle accelerated thrombosis of platelet and fibrin.

DS-87A model thrombus apparatus was used in experiment. 1 ml carotid blood was collected into a siliconated polyvinyl tube and putted into the rotating circle to imitate blood flow condition. After rolling 15 min the thrombus was poured out. Weight (wet wt) and length it. After drying (56°C , 22 h) weigh again (dry wt). The dry wt of thrombus in drug group and control group were compared statistically. Urokinase was used as positive control.

The determination of metamorphosis capability of RBC in human body

Metamorphosis capability of RBC was shown by IF (Index of Filtration). The less of IF value, the better of the metamorphosis capability of RBC is.

$$\text{Method of calculation: IF} = \frac{t_s - t_b}{t_b} \times \frac{1}{H}$$

t_s : The time of filtration of RBC suspension

t_b : The time of filtration of normal saline (not containing any RBC)

H: Hematocrit of RBC suspension, means the volume of RBC (%)

Plasma poor whole blood of human was incubated at 33°C for 36 h, and then in room temperature about 48 h. The RBC aged naturally and IF value increased significantly. The aged plasma poor whole blood was washed with NS, centrifuged, to remove WBC, etc. The remaining RBC was prepared about 5% RBC suspension and determined hematocrit (H) of it.

5% RBC suspension was divided and transferred into some tubes. 10 ml/tube for test. 800 μl normal saline was added into the control tube. The same volume of different concentrations of LZ I or LZ II were added into drug groups. The IF value of control group and drug group were determined in 15 ~ 30 min. If the IF of drug group < IF of control group, there were recovering effect of this concentration of the drug for aging RBC.

Results

Effect of LZ on experimental thrombosis in rats

The results showed that the weight of thrombus in three groups (control, Aspirin and LZ) were different statistically. LZ CMC solution 500mg/kg x 2 (po.) showed action against the platelet aggregation ($P < 0.01$), while there was no effect with 100 mg/kg x 2 (po.) (Tab. 20-1).

Tab. 20-1 Effect of LZ on Platelet aggregation in Rats

Group	Body Wt (g)	Wet Wt of Thrombus (mg)	P Value
0.2 M Phosphate Buffer iv. 1 ml/kg	322 ± 26 (9)	25.05 ± 3.25 (9)	
1.5% Aspirin iv. 30 mg/kg	330 ± 41 (9)	11.32 ± 2.78 (9)	< 0.01 (compared with buffer)
0.5% CMC solution po. 1 mg/kg x 2	340 ± 47 (8)	25.28 ± 4.66 (8)	
LZ CMC Solution po. 500 mg/kg x 2	320 ± 27 (8)	16.98 ± 2.04 (8)	< 0.01 (compared with CMC)
LZ CMC Solution Po. 100 mg/kg x 2	307 ± 28 (8)	21.51 ± 6.55 (8)	> 0.05 (compared with CMC)

$\bar{x} \pm SD$

The results showed as same as in vivo experiment. CMC suspension 500 mg/kg x 2 (po.) resulted the action against experimental thrombosis of rats. The dosage of 100 mg/kg x 2 (po.) did not show action at all (Tab. 20-2).

Tab. 20-2 Effect of LZ on Platelet and Fibrin Thrombosis in Rats ($\bar{x} \pm SD$)

Group	No. of Animals	Time of Platelet Thrombosis (s)	Time of Fibrin Thrombosis (s)	Length of Thrombus (mm)	Wt of Thrombus (wet) (mg)	Wt of Thrombus (dry) (mg)
Urokinase (1 500 μ /kg) iv.	10	163 \pm 43**	278 \pm 31*	11.0 \pm 1.2**	34.10 \pm 1.2**	8.39 \pm 0.95**
0.5% CMC Solution (10 ml/kg) x 2 po.	10	108 \pm 32	191 \pm 29	14.5 \pm 1.7	45.64 \pm 4.18	11.68 \pm 1.58
LZ CMC Suspension (500 mg/kg) x 2 po.	10	155 \pm 23**	273 \pm 32**	12.1 \pm 1.0**	28.30 \pm 5.39**	7.74 \pm 1.53**
LZ CMC Suspension (100 mg/kg) x 2 po.	8	147 \pm 28*	250 \pm 40**	13.3 \pm 1.1	40.34 \pm 5.84*	11.30 \pm 1.50

*P<0.05, **P<0.01 compared with CMC

Effect of LZ on metamorphosis capability of RBC in human body

The experiments revealed that LZ Solution I in the final concentration more than 10 mg/ml showed remarkable recovering effect for the metamorphosis capability of RBC (P < 0.01). The LZ Solution II showed the remarkable recovering effect in the final concentration 2.0 mg/ml (P < 0.05). The ultra wave suffering of LZ might increase the solubility of its effective elements (Tab. 20-3, 4).

Tab. 20-3 Effect of LZ Solution I on Metamorphosis capability of human RBC

Group	Control	LZ Solution I (mg/ml RBC Suspension)			
		5.0	10.0	20.0	25.0
IF ($\bar{x} \pm SD$)	0.52 \pm 0.06	0.46 \pm 0.05	0.38 \pm 0.06	0.31 \pm 0.04	0.31 \pm 0.02
	(9)	(9)	(9)	(9)	(6)
P		< 0.05	< 0.01	< 0.01	< 0.01

() no. of samples

Tab. 20-4 Effect of LZ Solution II on Metamorphosis capability of human RBC

Group	Control	LZ Solution II (mg/ml RBC Suspension)		
		1.0	2.0	4.0
IF ($\bar{X} \pm SD$)	0.44 \pm 0.05	0.45 \pm 0.05	0.39 \pm 0.05	0.39 \pm 0.05
	(14)	(8)	(9)	(9)
P		> 0.01	< 0.05	< 0.05

() no. of samples

Discussion

LZ has been used widely in clinic. It decreases blood pressure and triglyceride effectively and is useful for the treatment of hypertension, coronary heart disease and hyperlipidemia⁽¹⁾. Sometimes it was used in brain thrombus, with inhibiting platelet adhesion^(2~5), enhancing immune function^(6, 7), treating chronic hepatitis, dermatositis, multiple myositis⁽⁹⁾. Chronic bronchitis, asthma, arrhythmia, Keshan disease, psychasthenia, tumor, etc. Our experimental results reveal that LZ shows the action against thrombosis, increasing metamorphosis capability of RBC, in accordance with the results of above references. However there were no effect of LZ for blood coagulation.

Effects of Ling Zhi on Hemorrheology Parameters and Symptoms of Hypertension Patients with Hyperlipidemia and Sequelae of Cerebral Thrombosis

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NING Yihua** BAI Zhijuan** DONG Shuang***
YE Yuchan Masao MORI****

Abstract The effects of Ling Zhi (LZ, *Ganoderma Lucidum*, Fr. Karst) on hemorrheology parameters and symptoms of hypertensive patients with hyperlipidemia, sequelae of cerebral thrombosis and coronary heart disease were studied. The LZ extract was given to 33 patients (15 male, 18 female, 65 ± 8 years old) in dosage of 110 mg, qid, po. for 2 weeks. The results showed that whole blood viscosity (at high shear rate and low shear rate) and plasma viscosity were significantly decreased ($P < 0.01$). LZ showed the effect of reducing blood pressure simultaneously. Several symptoms were also improved. However, there were no significant changes in haematocrit and erythrocyte sedimentation rate ($P > 0.05$).

Key words Ling Zhi (LZ, *Ganoderma Lucidum*, Fr. Karst); Thrombosis; Hemorrheology.

LZ, a well-known and widely used Chinese traditional medicine, has been revealed to possess antihypertensive, antianginal and antilipidemic effects (1 ~ 3). In this paper, we report the effects of LZ on hemorrheology of 33 patients with high blood viscosity.

Subjects and Methods

Subjects for study

LZ group: 33 outpatients (15 male and 18 female; 45 ~ 86 years old, mean age 65 ± 8), including 12 hypertension with hyperlipidemia, 10 with sequelae of cerebral thrombosis, 5 with coronary heart disease, 5 with hyperlipidemia and 1 with recovery of myocardial infarction. Placebo group: 10 outpatients (4 male and 6 female, 52 ~ 76 years

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old, mean age 61 ± 7), including 8 hypertension with hyperlipidemia; 1 with sequelae of cerebral thrombosis and 1 with coronary heart disease.

Reagents

LZ tablets provided by Wakan Shoyaku Botany Institute, Tokyo, Japan. Each tablet contained 55 mg, dry powder extract of LZ, equivalent to 1.375 g of crude drug.

Treatment procedure

LZ extract was given orally in dosage of 110 mg qid for 2 weeks. One week before treatment, the medicines had to be given up except part antihypertensive drugs. The symptoms including dizziness, headache, tinnitus, asthenia, mental dullness, insomnia, hypomnesia, slurred speech and flaccid somatic muscle, the signs including blood pressure and pulse, and hemorrheologic parameters would be compared before and after treatment.

Evaluation

Marked effectiveness means patients without symptom or with more than 90% improvement. Effectiveness means more than 50% improvement. None-effectiveness means patients with original symptoms or less than 50% improvement.

Examinations

The hemorrheologic parameters included the whole blood viscosity (at high shear rate and low shear rate), plasma viscosity, haematocrit and erythrocyte sedimentation rate.

Equipment

LIANG – 100 type computerized glass capillary blood viscosimeter with a variable shear rate, made by Shanghai Medical University⁽⁴⁾.

Values are presented as mean and SD. Parameters before and after treatment of the same patients were compared for analyzing the data by using the student's t test.

Results

Clinical data

Improvement of some symptoms, such as headache, insomnia, dazzle, chest tightness and numbness in limbs, was observed in LZ group (Tab. 21-1). The effective rate was 75.0%, 64.7%, 58.8%, 53.8% and 52.9% respectively. However, no changes were observed in placebo group.

Hemorrhheologic variation

The blood viscosity was improved by using LZ. The whole blood viscosity was decreased at high shear rate in 26 and at low shear rate in 24 and plasma viscosity was reduced in 26 of the 33 patients. The improvement rate was 78.8%, 72.2% and 78.8%, respectively. Significant differences ($P < 0.01$) were observed before and after treatment with LZ (Tab. 21-2). Moreover, the haematocrit and erythrocyte sedimentation rate were not influenced by LZ. The variations in placebo group were insignificant.

Tab. 21-1 Changes in clinical manifestations after treatment with LZ for 2 weeks

	LZ					Placebo (for control)			
	Cases	ME	RE	IE	ER(%)	Cases	ME	RE	IE
Subjective Symptoms									
Dizziness	18	4	5	9	50	7	—	—	7
Carebaria	10	2	3	5	50	4	—	—	4
Headache	12	6	3	3	75	3	—	1	2
Dazzle	17	4	6	7	58.8	5	—	1	4
Tinnitus	11	2	3	6	45.5	5	—	—	5
Numbness in limbs	17	4	5	8	52.9	7	—	1	6
Asthenia	18	5	3	10	44.4	6	—	1	5
Chest tightness	13	5	2	6	53.8	4	1	—	3
Angina Pectoris	6	2	0	4	33.3	—	—	—	—
Psychic symptoms									
Mental dullness	11	2	0	9	18.2	4	1	—	3
Insomnia	17	10	1	6	64.7	7	3	—	4
Hypomnesia	18	4	1	13	27.8	6	—	—	6
Neurologic symptoms									
Slurred speech	8	1	1	6	25	1	—	—	1
Flaccid somatic Muscle	11	1	3	7	36.7	3	—	—	3

ME = marked effectiveness; RE – remission; IE – ineffective; ER = effective rate (including marked effectiveness and remission)

Tab. 21-2 Effects of LZ on hemorrheologic parameters

	LZ			Placebo (for control)		
	Cases	BT	AT	Cases	BT	AT
A	33	6.80 ± 0.59	6.37 ± 0.63**	10	6.99 ± 0.71	7.11 ± 1.23
B	33	11.75 ± 2.39	10.40 ± 1.97**	10	12.84 ± 3.63	12.42 ± 3.60
C	33	1.94 ± 0.13	1.86 ± 0.12**	10	1.89 ± 0.08	1.90 ± 0.14
D	33	46.81 ± 3.66	46.59 ± 3.53	10	45.65 ± 2.99	46.75 ± 3.10
E	33	22.65 ± 11.99	23.01 ± 11.24	10	24.55 ± 12.54	19.00 ± 12.24

A: whole blood relative viscosity at high shear rate (cP);

B: whole blood relative viscosity at low shear rate (cP);

C: plasma relative viscosity (cP);

D: Haematocrit (%);

E: erythrocyte sedimentation rate (mm/h);

$\bar{x} \pm SD$; BT = Before treatment, AT = After treatment for 2 weeks, ** P<0.01 compared with that before treatment.

Blood pressure and pulse

Blood pressure in 17 patients of hypertension without drugs was measured after receiving LZ. Systolic and diastolic pressures were markedly reduced (Tab. 21-3). It showed that LZ had antihypertensive action. No obvious change in pulse was observed in 33 patients.

Tab. 21-3 Effects of LZ on heart rate and blood pressure

	Cases	BT	AT
Heart rate (beats / min)	30	74.47 ± 9.68	77.07 ± 8.74
Systolic pressure (kPa)	17	19.58 ± 2.48	17.92 ± 2.85**
Diastolic pressure (kPa)	17	11.84 ± 0.96	10.98 ± 1.50**

$\bar{x} \pm SD$; BT = Before treatment, AT = After treatment for 2 weeks, ** P<0.01 compared with that before treatment.

Adverse reactions

Adverse reactions of LZ were rare. Palpitation was seen in 2 patients and restlessness in 1.

Discussion

Clinical trials made on 33 patients with hyperviscosaemia demonstrated that LZ decreased viscosity of whole blood and plasma, reduced blood pressure and improved some symptoms. The blood hyperviscosity which reduces the blood flow and tends to increase the incidence of thrombosis may be caused by many factors such as hyperglobulia, poor deformability of red blood cell, hyperactivity of platelet aggregation, hyperlipidemia etc. Some studies reported that LZ reduced the levels of triglyceride and cholesterol^(1,3), inhibited platelet aggregation induced by ADP and collagen⁽⁵⁾. It has been made clear that LZ possessed the actions against platelet thrombus and fibrin thrombus and recovered metamorphosis capability of aged red blood cells⁽⁶⁾.

As indicated above, the decrease of blood viscosity is due to multiple effects of LZ. LZ is a valuable agent to improve blood fluidity, increase blood supply and prevent thrombus formation. It may be used to prevent the development of cardiocerebrovascular disorder complicated by high blood viscosity.

Ling Zhi – An Immunomodulator (A Review)

ZHANG Luoxiu

The first description of Ling Zhi (LZ) like fungus can be traced back to 3 century BC in the ancient work of “Li Ji, Nei Ce and Iu Shi Chun Qiu”. Its effects had been written in more detail in 1 700 years ago. In the oldest Chinese practical textbook of medicine “Shen Nong Ben Cao Jing” it was recorded that LZ was a very precious drug. LZ was divided into 6 kinds according to the color possessed different activities. For 2 000 years LZ has been graded as the upper grade of drugs. It means LZ is non toxic and can be used for a long time without harmfulness. It has been regarded as a very good drug to keep healthy and long life being used widely in the folk medicine.

Chinese medicine was introduced into Japan between the 3rd to 8th centuries. The famous Chinese Buddhist priest Jian Zhen He Shang (Buddhist Monk Tian Zhen) came to Japan in 753 AD bringing with him a number of drugs through which he introduced Chinese medicine to Japan. From then on Chinese medicine was the mainstream of medicine and practiced extensively in Japan since the 16th, though how LZ came to Japan was still not clear. However, LZ is named as “Lucky Herb, Miraculous Herb, Auspicious Herb, Long Life Herb” etc. in the folk Japanese medicine reflected how important it is.

During the past 50 years LZ has gradually attracted the attention of scientists not only in Japan but also in other countries. It was recorded in “Fungus dictionary” published in 1983 that there were 113 strains in LZ family, 86 strains growing in China possessing more than 76% of strains in the world. Modern technique has been used to elucidate the differences in genes and biochemical studies within the strains of LZ family by the scientists in Taiwan. The first study of the chemistry analysis of LZ was reported by a Japanese scientist in 1958. Up to now more than 150 compounds have been isolated from LZ. There are polysaccharides, furanes, ribosides, peptides and triterpenes etc. Among these the pharmacology of polysaccharides was studied in more detail.

Hot water extract is the traditional preparation for Chinese medicine including LZ. In general it is a complex of a number of biologically active substances in which pharmacological actions may antagonize or synergy each other. However this may be natural phenomenon, considering the composition of human body which represents one species of living things. It was the traditional preparation bringing such favorite activities before although it has not been elucidated deeply.

The rapid advance in immunology has contributed a great deal to clarify the etiology and pathological mechanism related to immunological disorders. Immunopharmacology becomes a new discipline in pharmacology. It is an area to attract

attention world wide. People began to understand how to regulate the host immunodefense and immunosurveillance function for keeping the regulative net work in self balance. It may be one of the most effective therapy in 21 century. The questions are: what is the immunopharmacological activity of LZ? Is there any effect of this traditional precious drug on immune system? What is the basic mechanism of this drug to alleviate various patients' complaints, to protect health, to treat disorders and to be used for such a long time?

We cooperate with Wakan Shoyaku Botany institute, Tokyo, Japan to study the pharmacological activities of LZ.

The hot water extract of LZ (*Ganoderma Lucidum*, Fr. Karst) planted in Japan was studied in immunological field on the following aspects:

1. Effects on lymphocyte proliferation.
2. Effects on antibody productive cells and allergic reaction.
3. Immune suppressive effect in mice.
4. Effects on macrophage phagocytosis and carbon particles clearance test.
5. Influence on natural killer cells activities.
6. Effects on the production of Interleukin-1 (IL-1).
7. Effects on the production of interleukine-2 (IL-2).
8. Influence on the production of Tumor Necrosis Factor (TNF).
9. Effects on cardiac heterotopic transplantation.
10. Liver protective activities.
11. Effects on hemopoietic system.

From the contents mentioned above it was found that some activities of LZ not reported before were revealed. It was demonstrated that LZ was able to act on the immune system. It regulated the function of immune cells and immune factors which depended on the state of organism, the condition of immune system, the level of lymphocytes activated as well as the regime and dosage of drug. For example, about LZ on lymphocyte proliferation test in vitro, it was found that LZ directly stimulated the rest lymphocyte. After stimulation with suboptimal concentration of Con A, lymphocytes were slightly activated. LZ was able in synergy of the effect of suboptimal concentration of Con A on lymphocyte proliferation. However, when lymphocytes were highly activated by high

concentration of Con A LZ would antagonize the activity of Con A and inhibit lymphocyte proliferation. In general, low dosage of LZ may have stimulative activity and high dosage of LZ may have suppressive activity. But when the organism was highly activated by antigen, low dosage of LZ would produce suppressive effect.

It was found that the reaction of different type of cells in immune system to LZ was not the same. B cells seem more sensitive to be suppressed by LZ. Under the experimental conditions, LZ suppressed lymphocyte proliferation induced by Lps, decreased the number of PFC and reduced the level of hemolysin. The mechanism of LZ activity could be different in T cell and B cell. LZ stimulated macrophages phagocytosis both in vitro and in vivo by promoting the carbon clearance test and enhancing the function of reticuloendothelium system. LZ stimulated macrophages to secrete interleukin-1 (IL-1) and tumor necrosis factor (TNF). It was considered that LZ enhanced the immune defense mechanism. Both macrophages and natural killer cells are important in immune surveillance system. LZ stimulating the activities of macrophages and natural killer cells might increase the release of TNF and production of IL-2 from splenocytes. These should be considered to enhance the immune surveillance mechanism. LZ was able to inhibit hyperimmunity. For example, it could inhibit III and IV type hypersensitive reaction and also slightly suppress I type reaction.

Although LZ itself had no significant effect on the survival time of grafted heart muscle. No obvious influence could be detected when LZ in combination with optimal dosage of cyclophosphamide for cardiac heterotopic transplantation. Only in combination with suboptimal dosage of cyclophosphamide LZ showed a little increase survival time of heart muscle implanted. The mechanism was far from clear. However it was considered that not only the reactivity of host to transplanted antigen was inhibited, the anticoagulative and vascular dilative activity of LZ might also be evolved. The hepatoprotective activity and the activity of LZ to stimulate hemopoietic system were demonstrated through experimental model. The results suggested that LZ was able to modify the function of immune system. It looks like to act as an immunomodulator. It possessed two direction regulative effect which further elucidated the effect of LZ that had been simply but profoundly stated by the ancient medical scientist. It suggested that LZ might be able to stimulate immunodefense mechanism and enhance immunosurveillance mechanism. On the other hand, when the host was challenged by certain antigen causing hypersensitive reactivity, LZ might express immune regulative activity to suppress the hypersensitive reaction for keeping self-stable.

It should be emphasized that the study of LZ on immune system is just started. Some phenomena cannot be explained clearly. It is our opinion that LZ is not an elixir being able to cure all diseases. However, it expresses helpful pharmacological effects. There are many questions needed to be answered. Further study is necessary.

Many changes have already taken place in medicine during the second half of the 20th century, the most important of which is the occurrence observed in types of diseases. Statistical data on diseases show that the incidence of many infectious diseases which were once common globally has declined and that the chronic disorders have been increased instead, Under this status, traditional medicine including LZ attracting attention from world wide is reasonable.