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Effects of kappa-selenocarrageenan on membrane fluidity and ghost reseal ability of rat erythrocyte

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ABSTRACT Effects of kappa-selenocarrageenan (κ-SeC) on membrane fluidity and ghost reseal ability of rat erythrocyte were studied by measuring the fluorescence polarization and the NADH-cytochrome C oxidoreductase, respectively. After rats were given ig κ-SeC 140 mg·kg^{-1}·d^{-1}×30 d, the fluorescence polarization was decreased in comparison with saline control group ($P<0.05$). It suggested that κ-SeC increased the membrane fluidity. The reseal ability of erythrocyte membrane (ghost) was also elevated after ig κ-SeC 140 and 70 mg·kg^{-1}·d^{-1}×30 d ($P<0.05$).

KEY WORDS selenium; carrageenan; fluorescence polarization; erythrocyte membrane; blood viscosity; membrane fluidity; cytochrome C oxidase

Selenium, an essential trace element, possesses wide biological activities in the organism. Kappa-selenocarrageenan (κ-SeC) is an organic selenate, formed by partial substitution of Se for S in natural carrageenan[1]. Upon changing the inorganic Se into organic Se, its absorption becomes better and toxicity is lowered[2]. The Se bioavailability of κ-SeC in rabbits supplemented with κ-SeC was higher than that of Na_2SeO_3. The κ-SeC showed anti-arrythmic effects in experimental animals[3], and the Se possessed an effect of antioxygenation[4]. To probe κ-SeC practicability to be used as an antioxidant, we studied effects of κ-SeC on membrane fluidity and ghost reseal in rat erythrocytes.

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MATERIALS

Drugs κ-SeC containing 1.2% selenium (supplied by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100086, China) was prepared to the concentrations of 7, 3.5, and 1.75 mg·ml^{-1}, pH 6.5; Royal Jelly (RJ) was obtained from Shanghai First Chinese Medicine Works, 30 mg·ml^{-1}, lot № 91102101; 1,6-Diphenyl-1,3,5-hexatriene (DPH, lot № 267592 1188) from Fluka Co was prepared with tetrahydrofuran to the concentration of 2 mmol·L^{-1} and stored at 4 ℃, and diluted to 2 μmol·L^{-1} with phosphate-buffered saline 0.01 mol·L^{-1}(PBS) before use; NADH-cytochrome C and saponin were purchased from Sigma Co and E Merck, respectively.

Rats Wistar rats ($n=50$), 202±s 12 g, ♂ ♀ half/half, were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences.

Equipments Hitachi MPF-4 fluroscence spectrophotometer; Hitachi F-3000 fluorescence spectrophotometer; 721 spectrophotometer.

METHODS AND RESULTS

Rats ($n=50$) were randomly allocated into 5 groups, 10 (5 ♂, 5 ♀) in each. Drugs were given ig daily for 30 d respectively: 1) NS; 2) RJ 150 mg·kg^{-1}; 3) κ-SeC 140 mg·kg^{-1}; 4) κ-SeC 70 mg·kg^{-1}; 5) κ-SeC 35 mg·kg^{-1}.

Erythrocyte membrane fluidity

1 Preparation of membranes

Blood was obtained by decapitation and a

single-stage hemolysis method[5] in hypotonic solution was used for the preparation of erythrocyte membranes. Protein contents of the samples were assayed colorimetrically[6] (Tab 1).

Tab 1. Protein contents of erythrocyte membranes. n=10, $\bar{x}\pm s$. *P>0.05 vs NS group.

Group/mg·kg^{-1}	Protein contents/μg·ml^{-1}
NS	512±111
RJ 150	502±106*
κ-SeC 140	565±165*
κ-SeC 70	544±161*
κ-SeC 35	539±129*

The results showed that there was no significant difference among the groups.

2 Spectrogram of erythrocyte membranes labeled with DPH

Two ml of DPH 2 μmol·L^{-1} was added to 2 ml of "ghost" preparation and incubated at 25 ℃ for 30 min, Centrifuged at 2000×g for 10 min. The precipitate was washed with PBS 0.01 mol·L^{-1}, membranes were resuspended in 4 ml of PBS.

The spectra scanned by F-3000 fluroscence spectrophotometer showed maximal excitation and emission peaks at $\lambda_{ex}=362$ nm and $\lambda_{em}=432$ nm[7] (Fig 1).

3 Measurement of fluorescence polarization (P)

The polarization (P) of rat erythrocyte membrane fluorescence labelled with DPH for each group was determined[7] by MPF-4 fluorescence spectrophotometer ($\lambda_{ex}=362$ nm, $\lambda_{em}=432$ nm). The degree of polarization was calculated by:

$$P = (I_{VV} - GI_{VH}) / (I_{VV} + GI_{VH})$$

$$G = I_{HV} / I_{HH}$$

where I_{VV} and I_{VH} stood for the intensity of fluorescence, recorded at the analyzing orientations, ie when the emission light polarized

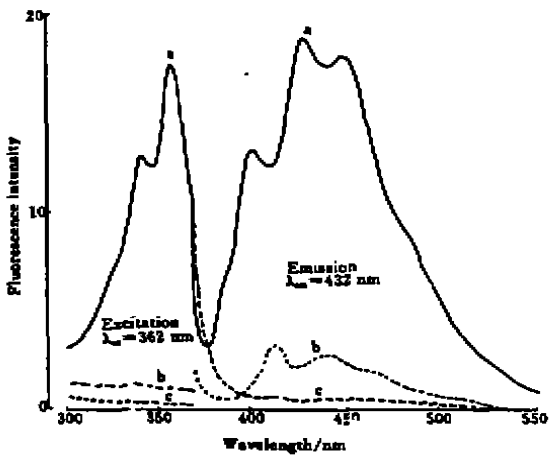

Fig 1. Excitation and emission spectrogram of erythrocyte membrane. Slit 5.5 nm, $\lambda_{em}=432$ nm, $\lambda_{ex}=362$ nm. a) Labeled with DPH, b) Unlabeled, c) DPH reagent.

with electric light vector vertically and horizontal. G denoted a correction factor. Since the emission passed through an analyzing monochrometer which itself had a polarizing effect, G could be used to correct the relative transmission of the emission monochrometer for the 2 polarization directions. I_{HH} was the intensity of emitted light when the 2 analyzing polarizer orientations were both at horizontal directions[8]. I_{HV} was the intensity of emission light when the emission polarizer at horizontal and the excitative polarizer at vertical orientation. The polarization (P) was the smaller, indicating lipid membrane fluidity was the larger.

As compared with liquid phase, a measure of the microviscosity was used to show the fluidity of membranes. From the following formula, the average microviscosity ($\bar{\eta}$) or anisotropy (r) was further calculated to represent the fluidity of membranes. Larger was the η or r, smaller was the lipid membrane fluidity.

$$\bar{\eta} = 2P / (0.46 - P)$$

$$r = 2P / (3 - P)$$

Tab 2. Fluorescence polarization (P), blood viscosity ($\bar{\eta}$) and anisotropy (r) of erythrocyte membranes. $n=10$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* NS group.

Group/mg·kg^{-1}	P	$\bar{\eta}$ ($\bar{\eta}$/protein)	r
NS	0.306±0.020	4.1±0.8 (80.2±9.1)	0.227±0.016
RJ 150	0.289±0.013^b	3.4±0.4 (69.5±11.6^a)	0.213±0.011^b
κ-SeC 140	0.27±0.03^c	3.0±0.9 (56.9±20.4^c)	0.199±0.024^b
κ-SeC 70	0.286±0.027^a	3.4±1.0 (69.5±31.5^a)	0.210±0.022^a
κ-SeC 35	0.303±0.028^a	4.1±1.4 (68.7±27.1^a)	0.225±0.028^a

The ratio of membrane $\bar{\eta}$ to the protein content in parentheses.

The polarization (P), the average microviscosity ($\bar{\eta}$), and the anisotropy (r) of rat erythrocyte membranes labelled with DPH was calculated by t test (Tab 2).

Results showed that κ-SeC ig 140 mg·kg^{-1} decreased the fluorescence polarization, average microviscosity and anisotropy of erythrocyte membrane.

Erythrocyte membrane reseal ability

1 Blood was anticoaguated with heparin and washed twice by NS to remove the WBC, platelets and fibrinogen. The washed red cells were suspended in cold PBS 5 mmol·L^{-1} (containing $MgSO_4$ 1 mmol·L^{-1}) pH 8.0, PBS : RBC = 40 : 1 (vol : vol). the erythrocyte ghost was isolated after 1 h by centrifugation at 20 000×g for 40 min, and washed twice again in PBS to remove the hemoglobin.

2 Activity of NADH-cytochrome C oxidoreductase was measured on the same sample with and without saponin[9]. After resealing, NADH-cytochrome C oxidoreductase was no longer measured. When saponin (0.1 % in PBS) was used to disrupt the permeability barrier, the maximal activity of the enzyme was shown. Reseal ability of ghost (impermeability) was calculated by: Impermeability = (Enzyme activity with saponin − Enzyme activity without saponin)/Enzyme activity with saponin × 100 %

The results showed that impermeability of erythrocyte membrane elevated after κ-SeC ig 140 and 70 mg·kg^{-1}·d^{-1}×30 d (Tab 3).

Tab 3. Reseal ability of erythrocyte ghosts. $n=10$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$ *vs* NS group.

Group/ mg·kg^{-1}	10^4×NADH-cytochrome C activity		Impermeability/%
	With saponin	Without saponin	
NS	95±64	45±28	53.1±5.0
RJ 150	99±46	42±18	59.8±3.5^b
κ-SeC 140	98±3	41±13	58.0±2.6^b
κ-SeC 70	97±5	42±13	57.0±2.1^b
κ-SeC 35	101±9	44±20	55.3±3.5^a

DISCUSSION

The membrane fluidity of erythrocyte is a physical feature indicating the flow of various membrane systems being similar to that in liquid state. When the fluidity decreases, the membrane proteins are more easily exposed to water solution and have an effect on the activity of enzymes[10]. The membrane fluidity of erythrocyte decreases in aging which is concerned with an increase in the saturated fatty acid contents[10]. So elevating the membrane fluidity did effect a beneficial action of antioxidation damage.

Membrane impermeability is operationally defined by the exclusion of specific probes, such as substrates, to the membrane enzymes. It was reported[11] that the membrane reseal ability of erythrocyte was relevant to the age

of cells. The membrane of erythrocytes lost the reseal ability with aging of the cells. For example, membrane reseal ability of young erythrocyte is about 92.9%. However, that of aged erythrocyte only 35.7%. From this view, drugs, which were able to elevate the membrane reseal ability of erythrocyte, possessed a positive effect of anti-oxidation damage. Because RJ can decrease the membrane microviscosity, increase the membrane fluidity and reseal ability, it was chosen as a positive control. The above studies showed that κ-SeC can decrease P, $\bar{\eta}$ or r values of erythrocyte membranes at a dose of 140 mg·kg^{-1}, increase the impermeability of erythrocyte membranes at a dose of 140 or 70 mg·kg^{-1}. The results suggested that κ-SeC was beneficial to the body regulating automatically and possessed some effect of antisenility.

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κ-硒化角叉菜胶对大鼠红细胞膜流动性与封闭度的影响

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A **摘要** 通过测定大鼠红细胞膜荧光偏振度 P 和 NADH-细胞色素 C 氧化还原酶的变化, 研究 kappa-硒化卡拉胶对红细胞膜流动性和封闭度的影响. 结果表明, kappa-硒化卡拉胶 140 mg·kg^{-1}·d^{-1}×30 d 可显著降低大鼠红细胞膜荧光偏振度, 即可显著提高细胞膜流动性 ($P<0.05$), ig 140 及 70 mg·kg^{-1}·g^{-1}×30 d 可显著提高大鼠红细胞膜封闭度 ($P<0.05$).

关键词 硒; 角叉菜胶; 荧光偏振度; 红细胞膜; 血液粘度; 膜流动性; 细胞色素氧化酶