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ORIGINAL RESEARCH

Protective effect of vitamin E on age-related alterations of Kupffer cell energy metabolism

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Abstract

AIM: To investigate the mechanism of age-related reduction of Kupffer cell (KC) phagocytic capacity and the protective management.

METHODS: Using rhodamine 123 fluorescence density and rate of glucose utilization as parameters, we measured the mitochondrial energy metabolism status *in vitro* and the glucose utilization capacity of isolated rat liver Kupffer cells (KCs) from rats of various ages (6 mo, 12 mo, 18 mo and 24 mo) and the effect of vitamin E (VE) pretreatment (500 mg/kg/wk × 13 wk).

RESULTS: The rate of KC glucose utilization and the rhodamine fluorescence density of KC mitochondria of 18 mo-old untreated rats (NVEG) were significantly lower than that of 6 mo-old NVEG by 19.3% (4.0 nmol·h ± 0.4 nmol·h^{-1} 10.6 cells^{-1} *vs* 5.7 nmol·h ± 0.6 nmol·h^{-1} 10^6 cells^{-1}, $P < 0.05$) and 19.5% (80.5 ± 6.3 *vs* 100.0 ± 4.7, $P < 0.01$) respectively; Rate of KC glucose utilization and the rhodamine fluorescence density of KC mitochondria of 6 mo-old rats were also lower than the 24 mo-old NVEG by 35.1% (3.7 nmol·h ± 0.6 nmol·h^{-1}10^6 cells^{-1} *vs* 5.7 nmol·h ± 0.6 nmol·h^{-1} 10^6 cells^{-1}, $P < 0.01$) and 32.1% (67.9 ± 7.4 *vs* 100.0 ± 4.7, $P < 0.01$) respectively. The two parameters of 18 mo-old VE pretreated rats (VEG) were significantly higher than those of 18 mo-old NVEG, and statistically comparable to those of 6 mo-old VEG. The two parameters of the 24 mo-old VEG were significantly higher in comparison with those of 24 mo-old NVEG, but still significantly lower than those of 6 mo-old VEG.

CONCLUSION: Aging has a significantly negative effect on KC energy metabolism, which can be alleviated by VE pretreatment.

Key words: Aging; Kupffer cells; Energy metabolism; Glucose metabolism; Vitamin E; Liver metabolism

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INTRODUCTION

Aged Kupffer cells (KCs) have a decreased phagocytic capacity[1]. A recent study found that alterations of cellular energy metabolism is one of the main causes responsible for the disordered cellular function[2]. The present study investigated the possible protective effect of vitamin E on the decrease in KC energy metabolism from aging in order to further study the mechanism of age-related suppressed phagocytosis, and a possible clinical intervention.

MATERIALS AND METHODS

Animal and groups

Forty healthy Wistar rats of both sexes and different ages were obtained from the Chinese Herb Research Institute of Sichuan Province. Rats were housed in climatized rooms on a 12 h light/dark cycle, with water and food *ad libitum* and were studied at 6, 12, 18 and 24 mo of age. Ten rats for each age group were divided at random into two groups, five treated with VE (VEG) and five that were not treated (NVEG), for a total of eight treatment groups with $n = 5$. Animals in VEG received an intraperitoneal injection of 5% VE solution for 3 mo (500 mg·kg·wk, 2 injections/wk) prior to the experiment. Animals in the NVEG groups received normal saline as control.

Isolation of KC

KCs were isolated using a collagenase perfusion method as previously described[1]. Briefly, after anesthesia (30 mg of barbital /kg body wt., intraperitoneally), the liver was perfused *in situ* with Ca^{2+} free Hanks balanced salt solution at 37 ℃ for 3 min. Then 0.05% collagenase (Type Ⅳ; Sigma) was added and the liver was perfused for an additional 4 min with Hanks balanced salt solution. The liver

Table 1 Rhodamine 123 fluorescence density of mitochondria and rate of glucose utilization of Kupffer cells of different age groups (n = 5, $\bar{x} \pm s$)

Group	Rhodamine 123 fluorescence density utilization of mitochondrion		Rate of glucose (nmol·h^{-1}·10^6 cells^{-1})	
	NVEG	VEG	NVEG	VEG
6	100.0 ± 4.7	100.0 ± 5.3	5.7 ± 0.6	5.6 ± 0.8
12	97.5 ± 7.1	99.4 ± 8.6	5.9 ± 0.8	6.0 ± 0.7
18	80.5 ± 6.3[b]	97.3 ± 6.8[d]	4.0 ± 0.4[a]	5.4 ± 0.5[d]
24	67.9 ± 7.4[b]	84.2 ± 8.7[bd]	3.7 ± 0.6[b]	4.5 ± 0.6[bd]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* 6 mo group; [d]$P < 0.01$ *vs* non-vitamin E group (NVEG). VEG: Vitamin E pretreated group.

was then removed and placed in buffer. Under a tissue culture hood, the liver was gently teased with a smooth forceps to release the cells and filtered through a 60 μm mesh. The cells were then centrifuged twice at 50 × *g* for 3 min to remove parenchymal cells (sediment). The supernatant was then sedimented at 300 × g for 10 min. The pellet of nonparenchymal cells was resuspended and cultured with RPMI 1640 medium (containing 15 mmol/L HEPES, 0.05 U/mL insulin, 15 mmol/L L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin) supplemented with 10% newborn calf serum. After 30 min, the non-adherent cells were removed. The viability of KCs was greater than 90% as determined by trypan blue exclusion.

Measurement of mitochondrial energy metabolism

The technique used to measure KC mitochondrial energy metabolism was a modification of that described previously[3]. Briefly, KCs were seeded on collagen-coated glass coverslips in 6-well plates at a density of 4 × 10^5 mL/well. After overnight culture, the wells were replenished with fresh medium, and supplemented with rhodamine 123 (Sigma) at a final concentration of 0.8 μmol/L. Cells were incubated at 37 ℃ for 15 min and then washed 3 times with RPMI 1640 medium. The coverslips were viewed with a fluorescence microscope (Olympus VANOX AHB-LB) using a 40 X objective. Excitation light for fluorescence was provided by a 100 watt lamp set at 490 nm. The fluorescence images were collected using a Panasonic CL320 videotape lens put into a computer image analyzing system (CMIAS007) and quantitative assessment of fluorescence was made using the computer software. Since rhodamine actively accumulates in mitochondria in response to changes in transmembrane potential, the amount of intracellular accumulation of this dye can be used to reflect the mitochondrial energy metabolism status.

Measurement of glucose utilization

Glucose utilization was measured using a modified method reported by Spolarics[4]. Briefly, KCs were seeded into a 24-well culture plate. The culture medium contained 20 mmol/L Hepes, 4.2 mmol/L $NaHCO_3$, 120 mmol/L NaCl, 5.4 mmol/L KCl, 1.8 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, 5 mmol/L glucose and 1% albumin with the pH adjusted to 7.4. After 24 h of culture, the supernatant was collected and stored at -20 ℃ until analysis. Glucose concentration was assayed enzymatically using oxidase and peroxidase.

Statistical analysis

Data were analyzed using variance analysis (ANOVA). When an ANOVA indicated a significant difference ($P < 0.05$), post hoc analysis was performed using Student-Newman-Keuls test.

RESULTS

The rate of KC glucose utilization and the rhodamine 123 fluorescence density of KC mitochondrion decreased with age (Table 1). These two factors were significantly lower in both 18 and 24 mo-old NVEG animals as compared to 6 mo-old NVEG rats. The rates of KC glucose utilization and the rhodamine fluorescence densities of KC mitochondria of 18 mo-old VEG were significantly higher than those of 18 mo-old NVEG, and statistically comparable to those of 6 mo-old VEG. These two factors were also significantly higher in 24 mo-old VEG animals when comparison with those of 24 mo-old NVEG rats, but still significantly lower than those of the 6 mo-old VEG animals.

DISCUSSION

Several studies[4] have demonstrated that macrophages readily oxidize glucose, and that glycolysis is an important source of energy in these cells. In physiological conditions, KCs are activated by endotoxins from the gut, and to some degree are a prerequisite for the regulation of some of the important functions of hepatocyte[5]. Therefore, a normal glucose utilization by KCs may be necessary to support liver's numerous metabolic and immunological functions. Therefore, it is of both theoretical and clinical significance to study the KC glucose utilization ability and mitochondrial energy metabolism status.

So far, very few reports are available on the KC mitochondrial energy metabolism. The reasons for this may include the complexity of the isolation process of KC, the limited number of KC recovered, and the difficulty of the mitochondria isolation, making it impossible to investigate KC mitochondrial respiratory function by the polarographic method usually used for hepatocytes' mitochondrial study. In this study, we examined the energy metabolism status of KC with rhodamine 123, as reported by Lemasters[3] originally used to investigate the energy metabolism of hepatocytes.

Rhodamine 123 uptake in living cells can be used as an indicator of mitochondrial membrane potential, since it has been reported that this fluorescent dye actively accumulates in mitochondria in response to changes in transmembrane potential[6]. In the absence of inhibition of the mitochondrial ATPase, the mitochondrial membrane potential should, more or less, be proportional to the phosphorylation potential, which is the free energy change of ATP synthesis. The present study demonstrated that this method was also a qualified one for the study of the mitochondrial energy metabolism of KC.

Oxidants are a major contributor to the aging process[7]. VE is the most effective chain breaking lipid-soluble antioxidant in the biological membrane, where it contributes to membrane stability. It protects critical cellular structures against damage from oxygen free radicals (an atom or group of atoms containing an unpaired electron) and reactive products of lipid peroxidation[8]. We used the VE supplementation dosage for the prevention of acute tissue injury reported elsewhere to determine the dosage used in the present study for the prevention or the treatment of ageing induced KC functional disorders.

The results of our study found that the rate of KC glucose utilization and the rhodamine 123 fluorescence density of KC mitochondrion decreased with age. These two factors were significantly lower in 18 and 24 mo-old untreated rats (NVEGs) than that of 6 mo-old NVEG. These data are further evidence of the aging theory of energy metabolism disorders. At the same time, these results also indicate that in the evolving course of Wistar rats, the KC age-related energy metabolism disorders occurred between the ages of 12 and 18 mo, a period equivalent to that of the ages of 40 and 55 years-old for human beings. These results also showed that the rates of KC glucose utilization and the rhodamine fluorescence densities of KC mitochondria of 18 mo old VEG rats were significantly higher than those of 18 mo-old NVEG rats and statistically comparable to those of 6 mo-old VEG. Additionally, those factors were significantly higher in 24 mo-old VEG rats as compared with those of 24 mo-old NVEG rats; however, they were still significantly lower than those of 6 mo-old VEG. From the data above we might as well infer that VE is effective in preventing the KC mitochondrial decay due to ageing, however it can only alleviate the already formed damage to some degree, indicating that the principle of "Prevention First" should be followed by the human being for the management of aging, which is in conformity with the principle of the "fight against aging should begin from childhood" in traditional Chinese medicine.

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