Effect of 3, 4-dihydroxyacetophenone on Na^+, K^+-ATPase activity of injured mitochondria and the oxygen consumption of brain cells of rat

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Abstract: Aim To investigate the effect of 3, 4-dihydroxyacetophenone (α-DHAP) on Na^+, K^+-ATPase activity of injured brain mitochondria induced by ascorbate-$FeSO_4$ and the oxygen consumption of rat brain cells stimulated by ADP. **Methods** Na^+, K^+-ATPase activity was determined according to the method of inorganic phosphate. Swelling of the brain mitochondria was detected with the method of spectrophotometer. Lipid peroxidation was detected according to the thiobarbituric acid method of spectrophotometer. Oxygen consumption was measured by oxygen electrode method. **Results** The decrease of Na^+, K^+-ATPase activity, mitochondria swelling and formation of lipid peroxidation were shown in rat brain mitochondria and cells induced by ascorbate-$FeSO_4$. α-DHAP was shown to increase the activity of Na^+, K^+-ATPase, decrease the mitochondria swelling and inhibit the production of lipid peroxidation of brain mitochondria and cells induced by ascorbate and $FeSO_4$. α-DHAP can also reduce the oxygen consumption of brain cells stimulated by ADP. **Conclusion** α-DHAP can protect the structure and the function of brain mitochondria and cells by scavenging the free radical and resisting the reaction of lipid peroxidation.

Key words: 3, 4-dihydroxyacetophenone; Na^+, K^+-ATPase; mitochondria swelling; lipid peroxidation; oxygen consumption

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α-青心酮对损伤的脑线粒体 Na^+, K^+-ATPase活性和脑细胞耗氧的作用

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摘要: 目的　研究 α-青心酮对抗坏血酸和硫酸亚铁诱导脑线粒体 Na^+, K^+-ATPase活性和脑细胞耗氧的作用。方法　采用无机磷法测定 Na^+, K^+-ATPase活性,分光光度法检测脑线粒体膨胀和脂质过氧化物,氧电极法测定脑细胞耗氧量。结果　在抗坏血酸和硫酸亚铁的作用下,鼠脑线粒体 Na^+, K^+-ATPase活性降低,线粒体膨胀和脑细胞脂质过氧化物升高。α-青心酮抑制其抗坏血酸和硫酸亚铁诱导脑线粒体和细胞的损伤,增加 Na^+, K^+-ATPase活性,降低脑线粒体膨胀和脑细胞脂质过氧化物生成。α-青心酮还具有减少 ADP刺激的脑细胞耗氧的作用。结论　α-青心酮通过清除自由基和抗氧化作用保护脑细胞结构和功能的完整。

关键词: α-青心酮; Na^+, K^+-ATP酶; 线粒体膨胀; 脂质过氧化物; 耗氧量

Much attention has been paid to the relationship between the pathological mechanism of cerebral injury and the reaction of oxygen free radicals[1]. The oxygen free radical is one of the main factors that leads to the reaction of lipid peroxidation, the injuries of cerebral membrane structure and function, and many kinds of

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diseases of the brain nerves, such as aging, Alzheimer, stroke *etc*. Therefore, it is of great significance in the study of the drug antioxidation in an attempt to prevent and treat brain neuron diseases[2].

α-DHAP is an effective component extracted from *Ilex pubescens* Hook. et Arn which is now used in clinic to treat coronary heart disease and cerebral thrombosis. Modern pharmacological research shows that α-DHAP has the effect of promoting blood circulation, removing blood stasis, and inhibiting platelet aggregation.

This paper observes, from the aspect of antioxidation, the effect of α-DHAP on injuries of the cerebral cells so as to provide a theoretical basis for its clinical application.

Materials and methods

Drug and reagents α-DHAP was obtained from the Institute of Pharmaceutical Research in Beijing, with the purity > 98%. 1, 1, 3, 3-Tetraethoxypropane was purchased from the Fluke Chemical Co. Bovine serum albumin, adenosine diphosphate (ADP) and the thiobarbituric acid (TBA) were provided by the Sigma Chemical Co. Ascorbate, $FeSO_4$ and other reagents with the highest procurable analytic purity were produced by Beijing Chemical Co..

Animals Male Wister rats (180 - 200 g) were provided by the Experiment Animal Center of Chinese Academy of Sciences.

Preparation of brain mitochondria Brain mitochondria were isolated according to the method as previously described[3]. The isolation medium contained 0.18 mol·L^{-1} KCl and 20 mol·L^{-1} Tris-HCl buffer (pH 7.4). The protein of mitochondria was determined by the Lowry′s method, using bovine serum albumin as standard.

Determination of Na^+, K^+-ATPase activity Na^+, K^+-ATPase activity of the mitochondria was determined based on the method of the paper[4]. The reaction mixture contained 100 mmol·L^{-1} NaCl, 20 mmol·L^{-1} KCl, 3 mmol·L^{-1} $MgCl_2$, 5 mmol·L^{-1} Na_2-ATP and 50 mmol·L^{-1} Tris-HCl (pH 7.4). The sample was analyzed for liberated inorganic phosphate, and Na^+, K^+-ATPase activity was expressed as μmol·mg^{-1} (protein)·h^{-1}.

Measurement of brain mitochondria swelling The swelling of brain mitochondria was measured according to the method of the paper[3]. The absorbance was determined at the wavelength of 520 nm of the spectrophotometer, and the decrease was taken as an index of the mitochondria swelling.

Determination of lipid peroxidation The lipid peroxidation was determined according to the method as previously described[4]. MDA μmol·g^{-1} (protein) was adopted to express the lipid peroxidation content, using 1, 1, 3, 3-tetraethoxypropane as standard.

Determination of oxygen consumption The oxygen consumption of brain cells was measured by the method of Alberto *et al*[3]. The reaction medium 2.0 mL (maintained at 30 ℃) contained brain cells (1 mg of protein), 0.225 mol·L^{-1} sucrose, 1.0 mmol·L^{-1} EDTA, 5 mmol·L^{-1} $MgCl_2$, 15 mmol·L^{-1} KH_2PO_4, 50 mmol·L^{-1} Tris-HCl, pH 7.2 with 10 μmol·L^{-1} *D, L*-malic acid plus 10 μmol·L^{-1} (+)-glutamic acid as respiratory substrate.

Statistical analysis Data were presented as $\bar{x} \pm s$. The differences among different groups were analyzed using the student′s *t*-test. A *P*-value less than 0.05 was considered as significant.

Results

1 Effect of α-DHAP on Na^+, K^+-ATPase activity of brain mitochondria induced by ascorbate-$FeSO_4$

The Na^+, K^+-ATPase activity of brain mitochondria was determined in the control group, the ascorbate-$FeSO_4$ induced group and the groups of ascorbate-$FeSO_4$ with various concentrations of α-DHAP. As a result, it was found that the 74.26% decrease of Na^+, K^+-ATPase activity was shown in the ascorbate-$FeSO_4$ group, compared with that of the control group. Compared with that of ascorbate-$FeSO_4$ group, there were 43.46%, 54.60% and 67.87% increase of the Na^+, K^+-ATPase activity when the reaction system presented at 4.81, 9.62 and 19.23 mmol·L^{-1} of α-DHAP, respectively (Table 1).

Table 1 Effect of 3, 4-dihydroxyacetophenone (α-DHAP) on the Na^+, K^+-ATPase activity of brain mitochondria

Group/mmol·L^{-1}	Na^+, K^+-ATPase /μmol·mg^{-1} (protein)·h^{-1}
Control	43.3 ± 1.9
Ascorbate-$FeSO_4$	11.2 ± 0.6
Ascorbate-$FeSO_4$ + α-DHAP 4.81	19.7 ± 1.1***
Ascorbate-$FeSO_4$ + α-DHAP 9.62	24.6 ± 1.2***
Ascorbate-$FeSO_4$ + α-DHAP 19.23	34.7 ± 0.8***

$n = 8$, $\bar{x} \pm s$. *** $P < 0.001$ *vs* ascorbate (100 μmol·L^{-1})-$FeSO_4$ (5 μmol·L^{-1}) group

Table 2 Effect of α-DHAP on brain mitochondria swelling (n = 3, $\bar{x} \pm s$)

Group/mmol• L^{-1}	A_{520}					
	0 min	5 min	10 min	20 min	40 min	60 min
Control	0.572 ±0.022	0.536 ±0.023	0.535 ±0.022	0.531 ±0.025	0.520 ±0.021	0.509 ±0.020
Ascorbate-$FeSO_4$	0.571 ±0.021	0.501 ±0.020	0.448 ±0.026	0.387 ±0.022	0.310 ±0.008	0.262 ±0.012
Ascorbate-$FeSO_4$ + α-DHAP 1.07	0.571 ±0.020	0.511 ±0.018	0.466 ±0.018	0.399 ±0.028	0.354 ±0.014	0.329 ±0.006
Ascorbate-$FeSO_4$ + α-DHAP 2.14	0.572 ±0.021	0.515 ±0.014	0.473 ±0.014	0.406 ±0.014	0.360 ±0.006	0.339 ±0.006
Ascorbate-$FeSO_4$ + α-DHAP 4.27	0.570 ±0.014	0.529 ±0.014	0.516 ±0.010	0.462 ±0.013	0.390 ±0.010	0.370 ±0.002

2 Effect of α-DHAP on brain mitochondria swelling

Effect of α-DHAP on brain mitochondria swelling-induced by ascorbate-$FeSO_4$ is shown in Table 2. The absorbance of brain mitochondria decreased significantly at 520 nm in ascorbate-$FeSO_4$ group (decreased 48.53% at 60 min). The absorbance of brain mitochondria increased to 35.36%, 33.40% and 27.31%, when the reaction solution presence of 1.07, 2.14 and 4.27 mmol• L^{-1} of α-DHAP compare with that of ascorbate-$FeSO_4$ group, respectively. The results indicate that α-DHAP is able to prevent brain mitochondria against swelling induced by ascorbate-$FeSO_4$.

3 Effect of α-DHAP on lipid peroxidation of brain cells

The experiment result showed an increase of lipid peroxidation production in the reaction system of brain homogenate induced by ascorbat-$FeSO_4$, but a decrease of lipid peroxides production in this reaction system when there was α-DHAP. The lipid peroxidation decreased from (14.1 ±0.8) μmol• mg^{-1} (protein) in control group to (11.2 ±0.6), (9.8 ± 0.5), (5.2 ± 0.3), (1.9 ± 0.4) μmol• mg^{-1} (protein) in the group of 1.60, 3.20, 6.41 and 12.82 mmol• L^{-1} of α-DHAP, respectively (Table 3).

Table 3 Effect of α-DHAP on the lipid peroxidation of brain cells

Group/mmol• L^{-1}	MDA/μmol• g^{-1} (protein)
Ascorbate-$FeSO_4$	14.1 ±0.8
Ascorbate-$FeSO_4$ + α-DHAP 1.60	11.2 ±0.6***
Ascorbate-$FeSO_4$ + α-DHAP 3.20	9.8 ±0.5***
Ascorbate-$FeSO_4$ + α-DHAP 6.41	5.2 ±0.3***
Ascorbate-$FeSO_4$ + α-DHAP 12.82	1.9 ±0.4***

n = 12, $\bar{x} \pm s$. *** P < 0.001 *vs* ascorbate (100 μmol• L^{-1})-$FeSO_4$ (5 μmol• L^{-1}) group

4 Effect of α-DHAP on oxygen consumption

Increased oxygen consumption was found in brain cells stimulated by ADP. However there was a decrease of oxygen consumption when various concentrations of α-DHAP were added into the system (Table 4).

Table 4 Effect of α-DHAP on oxygen consumption of brain cells

Group/mmol• L^{-1}	Oxygen consumption /μmol• mg^{-1} (protein)
ADP	72 ±10
ADP + α-DHAP 0.40	64 ±11*
ADP + α-DHAP 0.80	53 ±3***
ADP + α-DHAP 1.60	43 ±6***

n = 9 - 12, $\bar{x} \pm s$. * P < 0.05, *** P < 0.001 *vs* ADP (5 mmol• L^{-1}) group

Discussion

Protective effect of α-DHAP on the structure and function of brain mitochondria and cells was demonstrated by increased Na^+, K^+-ATPase activity, and decreased mitochondria swelling and lipid peroxidation.

The relationship between the mechanism of action for brain cell damage, and the free radical and lipid peroxidation reaction has been investigated widely in scientific research. The study of Chakraborty *et al*[5] indicated that iron promoted the production of $\cdot$OH free radicals, which induced the damage of the brain cells. Recent studies have demonstrated a possibility that the decreased Na^+, K^+-ATPase activity is related to the neuronal necrosis, under the condition when there was a hypoxic cerebral ischemia[6]. Oxygen-free radical-mediated lipid peroxidation has been proposed as one of the major mechanisms of cell membrane damage and inactivation of Na^+, K^+-ATPase[7].

Our previous studies showed that addition of ascorbate-$FeSO_4$ to the rat brain mitochondria or homogenate can cause decrease of Na^+, K^+-ATPase activity, and increase of lipid peroxidation. We also investigated the mechanism of action of the formation of $\cdot$OH free radical and lipid peroxides reaction induced

by ascorbate-$FeSO_4$. We found that dehydration of ascorbate might lead to increase of H_2O_2 at suitable environment and when H_2O_2 reacted with Fe^{2+} it would produce $^{\cdot}OH$ free radical, and $^{\cdot}OH$ free radical might react with brain cells membrane lipid polyunsaturated fatty acid, producing the lipid peroxidation, and resulting in the decrease of Na^+, K^+-ATPase[3].

α-DHAP is a kind of strong anti-oxidant which can increase the Na^+, K^+-ATPase activity under the condition when the brain cell damage is induced by ascorbate-$FeSO_4$. α-DHAP can increase the Na^+, K^+-ATPase activity through inhibiting the free radical reaction and the propagated chain of lipid peroxidation in the membrane of mitochondria initiated by ascorbate-$FeSO_4$.

The results of this experiment also confirmed that ascorbate-$FeSO_4$ can induce swelling of brain mitochondria. This is why the relationship between free radical reaction and mitochondria swelling was studied extensively. The substances leading to mitochondria swelling can also lead to the increase of lipid peroxidation. Since the peroxidation of mitochondria membrane lipid changes the liquid crystalline state of membrane lipids, and increases the permeability of the membrane, mitochondria swelling occurs[8]. The normal brain cell is present to protect the system by eliminating the free radicals, but under the condition of aging or diseases, the activity of the antioxidant enzymes reduced[9]. Therefore, certain kind of antioxidant should be supplemented. α-DHAP is the ideal drug which can increase the activity of the antioxidant enzymes and prevent the brain mitochondria swelling.

One of the main functions of the mitochondria in energy metabolism is oxidative phosphorylation, during the process of which there will be an increase of oxygen consumption, and accompanying increase of superoxide anions free radicals ($O_2^{\cdot}$). Our research confirmed that α-DHAP could decrease the oxygen consumption in oxidative phosphorylation, and prevent the formation of superoxide anions free radicals. Then the structure and the function of brain mitochondria and cell membrane can be effectively protected.

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