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中国人群 *N*-乙酰转移酶多态性的基因分析[1]

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关键词 *N*-乙酰转移酶; 多态性(遗传学); 基因型; 中国人; 等位基因; 聚合酶链反应

目的: 研究中国人群 *N*-乙酰转移酶(NAT2)多态性的基因基础. **方法**: 对 120 名汉族健康志愿者进行基因型分析. NAT2 基因型的检测应用等位基因 PCR 技术. **结果**: NAT2 基因的野生型及 M1, M2, M3 突变等位基因在 120 人中的发生率(WT = 0.625, M1 = 0.0458, M2 = 0.188, M3 = 0.142)存在显著差异($P < 0.01$), 由它们组合而成的各种基因型在人群中的分布符合 Hardy-Weinberg 平衡($\chi^2 = 7.27$, $\nu = 8$, $0.7 > P > 0.5$). 经过基因型分析可将受试者分为野生型纯合子、杂合子及突变纯合子, 其比例为 50:50:20. **结论**: 与高加索人比较, 中国人的 M1 突变基因的发生率较低, 说明了中国人慢乙酰化者比例较低的原因.

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Effects of vitamin C on myocardial mitochondrial function and ATP content in hypoxic rats

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KEY WORDS ascorbic acid; anoxia; heart mitochondria; adenosine triphosphate; blood flow velocity; blood gas analysis; blood pressure; membrane fluidity; Na^+-K^+-exchanging ATPase; myocardium

AIM: To observe the effects of large dose of vitamin C (V_c) on myocardial mitochondrial function, ATP content, and myocardial structure in acute and chronic hypoxic rats. **METHODS**: Rats were exposed to a simulated altitude 4000 m (barometric pressure = 43 kPa) for 3 and 30 d. V_c (0.75 g·kg^{-1}·d^{-1}) was injected ip. The heart mitochondrial respiratory function were determined by Clark-type O_2 electrode; mitochondrial membrane fluidity (MMF) were assayed through fluorescence polarizative method; the contents of ATP, ADP, and AMP in myocardial tissue were measured with HPLC. **RESULTS**: After administration of V_c, the ATP content was increased from 35 ± 3 mg·g^{-1} to 53 ± 3 mg·g^{-1} in acute hypoxic rats ($P < 0.01$), from 42 ± 4 mg·g^{-1} to 48 ± 3 mg·g^{-1} in chronic hypoxic rats ($P < 0.01$); P_{a,O_2} was increased from 7.2 ± 1.4 kPa to 9.5 ± 1.2 kPa in acute hypoxic rats ($P < 0.01$); mitochondrial respiratory control rate (RCR) was increased from 2.1 ± 0.6 to 4.7 ± 0.5 in acute hypoxic rats ($P < 0.01$), and from 3.3 ± 0.7 to 4.5 ± 0.6 in chronic hypoxic rats ($P < 0.01$); MMF was increased in acute and chronic hypoxic rats ($P < 0.05$); the degree of myocardial necrosis in vitamin C preventive rats was attenuated as compared with those of acute

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hypoxic rats. **CONCLUSION**: V_c is effective on improving myocardial energy metabolism and protecting against myocardial structural injury in hypoxic rats.

Myocardial energy metabolism failure caused by hypoxia is associated with the cellular Ca^{2+} overload, oxygen free radical, and the decrease of Na^+/K^+-ATPase activity[1-4]. Mitochondria which is sensitive to injury caused by free radicals played an important role in energy metabolism. The decrease in mitochondrial membrane fluidity caused by mitochondrial membrane injury may reduce the mitochondrial function and the activity of enzyme inlaying in membrane[5]. Vitamin C (V_c) is an oxygen derived free radicals scavenger[6], and has been successfully used to prevent mitochondrial injury in ischemic-reperfused myocardium[7,8]. The level of blood lipid peroxidate (LPO) was decreased in high altitude hemorrhagic shock goats treated with V_c[9]. However, it is little known whether V_c can protect the mitochondrial membrane structure and function, and improve myocardial energy metabolism during hypoxia. The aim of this study was to observe the effects of V_c on myocardial mitochondrial function and ATP content during hypoxia, and to assess whether V_c could prevent against hypoxic myocardial structural injury.

MATERIALS AND METHODS

Agents Sodium glutamate (Huzhou Biochemical Reagent Plant, China); Sodium *dl*-malate (Shanghai No 2 Chemical Reagent Plant, Shanghai, China); 1, 6-diphenyl-1, 2, 3-hexatriene (Sigma); 8-anilino-1-naphthalene-sulfonic (ANS) fluorescence probe (Fluka); ADP (Sigma); ATP (Sigma).

Rats Wistar rats of either sex ($n = 50$), weighing 220 ± 30 g (animal Grade Ⅰ, standard number: 24303015, from Experimental Animal Center of Third Military Medical University) were randomized into 5 groups: (1) normal control group in normoxic surrounding; (2) acute hypoxia (AH) in a hypobaric chamber simulated altitude of 4000 m for 72 h; (3) AH + V_c group was exposed in a hypobaric chamber simulated high altitude of 4000 m for 72 h (decompression condition was the same as that of group 2, these rats received V_c 0.75 g·kg^{-1}·d^{-1} for 3 d, ip); (4) chronic hypoxia (CH) group in a hypobaric chamber exposed to a simulated high altitude of 4000 m for 30 d (decompression condition was the same as that of group 2; (5) CH + V_c group was exposed to a simulated high altitude of 4000 m for 30 d (decompression condition was the same as that of group 2, these rats received V_c 0.75 g·kg^{-1}·d^{-1} for 30 d, ip).

Hemodynamic and blood gas measurement Rats were anesthetized with pentobarbital sodium 30 mg·kg^{-1}, ip. A catheter was placed in right ventricle and the right ventricle systolic pressure (RVSP) was measured with 8-channel physiologic recorder (RM-6000, Nihon Kohden, Japan). Another catheter was placed in the left carotid artery for blood gas analysis. Heart tissue (0.2 g) was preserved into liquid nitrogen for measuring the ATP content. Mitochondria was isolated from heart tissue. Another part was fixed in 10 % formalin. Measurements for normal control group were done at sea level, whereas the remaining groups were done at a simulated high altitude of 4000 m.

Mitochondria isolation[10] and respiratory function assay[11] The substrates were sodium *dl*-malate and sodium glutamate.

ATPase activity[5] and ANS fluorescence measurement[12] The total reaction volume was 500 μL, and 25 μL mitochondria were added.

Mitochondrial membrane fluidity assay[5] 1, 6-Diphenyl-1, 2, 3-hexatriene was used as a fluorescence probe.

Content of myocardium adenine nucleotides measurement[13] The HPLC system (Gilson, France), UV spectrophotometer (Gilson, France), λ 254 nm, sensitivity 0.01 AUFS, liquid phase PBS 2 mmol·L^{-1} (pH 5.5), 20 μL myocardium tissue.

Morphology After solidified with 10 % formalin, heart tissue was stained with hematoxylin basic fuchsin picric acid.

Statistical analysis Data were expressed as $\bar{x} \pm s$, compared with one-way ANOVA (pda-

2) using F-protected least significant difference test (FLSD).

RESULTS

Blood gas parameters and RVSP p_{a,O_2}, p_{a,CO_2} were decreased and RVSP was increased in acute and chronic hypoxic groups as compared with control group. V_c increased p_{a,O_2} in acute hypoxic rats and decreased the RVSP in acute and chronic hypoxic rats. There was no significant difference in pH between 5 groups (Tab 1).

Myocardial mitochondrial function Mitochondrial Ⅲ state O_2 consumption (ST_3), respiratory control rate (RCR) and ADP/O were apparently decreased and Ⅳ state O_2 consumption (ST_4) increased in acute and chronic hypoxic groups *vs* control group. V_c improved all above mentioned parameters (Tab 1).

Myocardial mitochondrial ATPase activity and ANS F_0F_1-ATPase, Na^+/K^+-ATPase activity, and ANS fluorescence intensity were significantly decreased in acute and chronic hypoxia group. All above mentioned parameters were improved obviously after treated with V_c (Tab 1).

Myocardial mitochondrial membrane fluidity Myocardial mitochondrial polarization degree and anisotropyin in acute and chronic hypoxic groups were increased *vs* control group, after treatment with V_c, though mitochondrial polarization degree and anisotropy were decreased, they did not return to the control level (Tab 1).

Myocardial adenine nucleotides The myocardial ATP content was decreased and while ADP and AMP contents were increased in all hypoxic rats. After treatment with V_c, the ATP content was increased while ADP and AMP contents were decreased (Tab 1).

Myocardial structure The myocardial fiber of the necroses dyed red, Whereas the normal myocardium dyed yellow. A lot of the distinct focal necroses in the myocardium occurred in all hypoxic rats. Sometime a piece of fusional necroses was seen in the endocardium (Fig 1). The focal necroses in the myocardium was attenuated in the acute hypoxic rats treated with V_c.

DISCUSSION

We proved that p_{a,O_2} was apparently reduced and RVSP was progressively increased in both hypoxic rats. After administration of V_c, the p_{a,O_2} was increased obviously and RVSP was apparently decreased in AH rats. RVSP is

Tab 1. Effects of V_c on biochemistry parameters in each group. $\bar{x} \pm s$.
$^bP<0.05$, $^cP<0.01$ *vs* control; $^eP<0.05$, $^fP<0.01$ *vs* acute hypoxia; $^hP<0.05$, $^iP<0.01$ *vs* chronic hypoxia.

	n	Control	AH	AH + V_c	CH	CH + V_c
p_{a,O_2}/kPa	10	14.6 ± 1.9	7.2 ± 1.4^c	9.5 ± 1.2^{cf}	7.2 ± 0.9^c	8.0 ± 1.1^c
p_{a,CO_2}/kPa	10	4.35 ± 0.14	3.24 ± 0.27^c	3.58 ± 0.12^c	3.38 ± 0.35^c	3.34 ± 0.26^c
pH	10	7.39 ± 0.06	7.41 ± 0.02	7.39 ± 0.05	7.38 ± 0.03	7.39 ± 0.02
RVSP/kPa	10	3.54 ± 0.24	5.55 ± 0.32^c	4.58 ± 0.28^{cf}	6.39 ± 0.36^{cf}	5.06 ± 0.38^{ei}
ST_3 (nanoatoms oxygen/mg·min)	10	332 ± 17	262 ± 21^c	311 ± 19^{cf}	265 ± 17^c	297 ± 22^{cf}
ST_4 (nanoatoms oxygen/mg·min)	10	61 ± 8	127 ± 6^c	66 ± 5^f	81 ± 6^{cf}	60 ± 8^i
RCR	10	5.4 ± 0.4	2.1 ± 0.6^c	4.7 ± 0.5^{cf}	3.3 ± 0.7^{cf}	4.5 ± 0.6^{ei}
ADP/O (nanoatoms oxygen/mg·min)	10	2.83 ± 0.17	2.20 ± 0.16^c	2.56 ± 0.09^{cf}	2.40 ± 0.11^c	2.60 ± 0.13^{ei}
F_0F_1-ATPase (μmolPi/mg·min)	10	7.17 ± 0.27	4.28 ± 0.18^c	6.60 ± 0.30^{cf}	5.42 ± 0.23^{cf}	6.01 ± 0.09^{ei}
Na^+/K^+-ATPase (μmolPi/mg·min)	10	1.36 ± 0.17	0.71 ± 0.12^c	1.09 ± 0.11^{cf}	0.92 ± 0.12^c	1.03 ± 0.13^c
ANS fluorescence intensity (relative unit)	10	54.5 ± 1.3	42.6 ± 1.0^c	47.7 ± 1.2^{cf}	43.9 ± 1.9^c	46.2 ± 1.7^{ei}
Polarization degree	9	0.232 ± 0.007	0.273 ± 0.005^b	0.256 ± 0.009^{be}	0.277 ± 0.011^b	0.259 ± 0.010^{bh}
Anisotropy	9	0.167 ± 0.008	0.200 ± 0.009^b	0.187 ± 0.010^{be}	0.200 ± 0.010^b	0.188 ± 0.007^{bh}
ATP/mg·g^{-1}	10	71 ± 5	35 ± 3^c	53 ± 3^{cf}	42 ± 4^{cf}	48 ± 3^{ei}
ADP/mg·g^{-1}	10	93 ± 9	115 ± 6^c	75 ± 7^{cf}	107 ± 8^{cf}	78 ± 12^{ei}
AMP/mg·g^{-1}	10	14.0 ± 2.3	19.9 ± 2.9^c	16.2 ± 2.7^f	19.0 ± 1.8^c	15.8 ± 1.6^i

Fig 1. Right ventricular myocardium from rats exposed to 4000 m for 3 d. HBFP stain, × 100.
A) Myocardial myofibril fusional necroses was shown with arrow. B) Treated with vitamin C 0.75 g·kg^{-1}·d^{-1} for 3 d.

equal to or slightly higher than pulmonary artery systolic pressure. Therefore, V_c may dilate pulmonary artery and improve pulmonary ventilation-perfusion ratio which was benefitial to increase of p_{a,O_2} in AH rats. A long-term hypoxia caused pulmonary blood vessel remodeling which led to the restriction of pulmonary blood vessel diastolic function[14]. The RVSP was slightly decreased in CH rats, so the variations of p_{a,O_2} is not apparent.

The myocardial mitochondrial F_0F_1-ATPase activity and ANS fluorescence intensity were obviously decreased which related to the decrease in mitochondrial membrane fluidity of both hypoxic rats. After administration of V_c, all above mentioned parameters recovered respectively. The increase of free radical production caused by ischemia and hypoxia is probably the most important mechanism in mitochondrial membrane injury[3]. As a result

of an radical scavenger, V_c is able to protect the mitochondrial structure and maintain the activity of enzyme inlaying in membrane.

Hypoxia caused the decrease in ST_3 and an increase in ST_4, which led to the decrease in RCR and ADP/O, the results were similar to that reported by Castar[15]. The decrease of ST_3 is related to the decrease of p_{a,O_2}, which led to the slow of electron transportation on respiratory chain. The increase in ST_4 is due to the increase in ineffective oxygen consumption caused by mitochondrial membrane injury. After prevented with V_c which not only partly recover the p_{a,O_2} but also protect the mitochondrial membrane in AH rats, then the ST_3 was increased and ST_4 was decreased leading to the recovery of RCR, ADP/O, and ATP production. On the other hand, due to the V_c did not result in any significant change of p_{a,O_2}, but only could increase MMF, so the increase in RCR and ADP/O of CH rats is mainly dependent on the decrease in ST_4 rather than the increase in ST_3.

In summary, V_c is an effective agent to improve mitochondrial energy metabolism, and prevent against hypoxic myocardial structural injury.

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维生素 C 对缺氧大鼠心肌线粒体功能和 ATP 含量的影响

R594·305

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关键词 抗坏血酸；缺氧症；心脏线粒体；腺苷三磷酸；血流速度；血气分析；血压；膜流动性；Na^+-K^+-交换 ATP 酶；心肌

目的：观察大剂量维生素 C (V_c)对缺氧大鼠心肌线粒体功能、ATP 含量及心肌结构的影响. **方法**：4000 m 高原停留 3、30 d，V_c(0.75 $g \cdot kg^{-1} \cdot d^{-1}$)腹腔注射. 线粒体呼吸功能测定采用 Clark 氧电极法；荧光偏振法测定线粒体膜流动性(MMF)，高效液相色谱测定心肌组织 ATP 含量. **结果**：应用 V_c 后，急、慢性缺氧组 ATP 含量、线粒体呼吸控制率显著增加($P < 0.01$)；MMF 明显恢复($P < 0.05$)；其中，急性缺氧组 p_{a,O_2} 明显上升($P < 0.01$)；心肌损伤程度降低. **结论**：V_c 可有效改善缺氧大鼠心肌能量代谢，对抗缺氧心肌损伤.