424-426 (11)

BIBLID : ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 Sep; **14** (5) : 424—426

红景天甙对培养心肌细胞缺氧后再给氧损伤的影响

叶于聪，陈钦铭，金凯平，周生祥，柴凤玲，海 平 （青海省高原医学科学研究所，西宁810012，中国）

R965.2 细胞培养

Effect of salidroside on cultured myocardial cells anoxia/reoxygenation injuries

YE Yu-Cong, CHEN Qin-Ming, JIN Kai-Ping, ZHOU Sheng-Xiang, CHAI Feng-Ling, HAI Ping (*Qinghai Institute of Plateau Medical Science, Xining 810012, China*)

ABSTRACT The effects of salidroside (*p*-hydroxyphenethyl glucoside, Sal, first isolated and synthesized in China) on reoxygenation damages were studied on cultured myocytes from neonatal rat hearts. At least 80% of cells in the form of monolayer contracted spontaneously on cultured 72 h, then the cells were used in the contractility experiment. After anoxia 3 h and reoxygenation for 1 h the beating of muocardial cells was slowed down and the lactate dehydrogenase (LDH) liberated by myocardial cells was increased. Electron microscopy of myocardial cells revealed localized defects of cell membrane, dilatation of endoplasmic reticulum, and swelling of mitochondria. One h before anoxia, addition of Sal 10 and 30 $\mu g \cdot ml^{-1}$ increased the beat rate of myocardial cells, depressed the release LDH of from myocytes, and the myocardial ultrasturcture was normal during anoxia and reoxygenation. Hence Sal may provide some protective effects on the anoxia/reoxygenation damages upon myocardium.

KEY WORDS salidroside; myocardium; cultured cells; anoxia; lactate dehydrogenase; electron microscopy; scanning electron microscopy

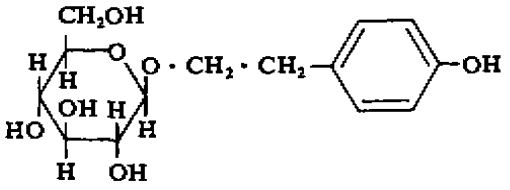
A

摘要 缺氧3 h 再给氧1 h 可引起培养的心肌细胞搏动频率减慢，乳酸脱氢酶(LDH)释放量增高，心肌细胞超微结构显示明显损害。红景天甙 (Sal) 10 及 30 $\mu g \cdot ml^{-1}$可使缺氧后再给氧心肌细胞搏动频率维持正常，减少 LDH 释放，心肌细胞膜、肌原纤维、线粒体等超微结构正常。表明 Sal 对缺氧后再给氧损伤心肌细胞有保护作用。

Received 1991-02-23 Accepted 1993-01-03

关键词 红景天甙；心肌；培养的细胞；缺氧；乳酸脱氢酶；电子显微镜检查；扫描电子显微镜检查

将大鼠由平原地区快速运抵海拔高3800 m地区，观察到某些红景天属植物对低氧所致在体心肌细胞损害具有保护作用[1]，并可提高心肌缺氧耐力[2]。红景天甙(salidroside，Sal)是首先从中国高山红景天(*Rhodiola sachalinensis* A Bor)中分离的，它是某些红景天属植物所共有的成份，它是否有可能为红景天属植物抗缺氧的有效成份，有待证明。为证实此设想，并寻找在高原地区需要的抗缺氧药物，我们进行了本研究。本文首次报道 Sal 对缺氧后再给氧损伤心肌细胞的保护作用及超微结构的影响。



Salidroside (*p*-hydroxyphenethyl glucoside)

MATERIALS AND METHODS

Sal (对羟基一苯乙基葡萄糖苷 *p*-hydroxyphenethyl-glucoside)合成品，由北京航天医学工程研究所提供，Sal 含量不少于99%，临用前用双蒸水配制成0.02%浓度，经 Scitz 滤器过滤消毒后备用。维拉帕米(verapamil，Ver 2.5 $mg \cdot ml^{-1}$)上海第十制药厂生产。

出生1—4 d 的 Sprague-Dawley 大鼠36只，♀♂兼用，本所动物房提供。按文献[4]方法制备心肌细胞悬液，细胞浓度为$1 \times 10^6 \cdot ml^{-1}$。培养液用含15%小牛血清的 M-199液。

实验方法 将上述心肌细胞悬液分装为每培养瓶含2×10^6个细胞，置37℃培育72 h，用 AO-1820型倒置

生物显微镜配合 XB-3型细胞搏动放大器，连接 XWT 台式自动平衡记录仪，描记细胞搏动频率与振幅变化. 80%以上心肌细胞形成单层，并以80 bpm 规则地成群搏动. 然后改换99.9% N_2的缺糖 Eagle 培养基2 ml (pO_2<4.00 kPa)培养3 h. 然后再换用含糖的 Hank's 液2 ml，再给氧1 h. 于改换高纯氮缺氧前1 h 分别加入不同浓度的 Sal 及 Ver 0.1 ml. 实验将32瓶缺氧后再给氧心肌细胞随机分成4组：对照组(加双蒸水)，Sal 10及30 μg·ml^{-1}组，Ver 46.6 μg·ml^{-1}组. 上述各组加入药物后5 h，观察心肌细胞的搏动. 释放 LDH 的浓度及光镜下形态改变. 随即以无 Ca^{2+}，Mg^{2+}的 Hank's 液洗涤细胞2次，再用2.5%戊二醛固定3—4 h. 然后将贴壁细胞刮下，置4℃离心 (800×g) 20 min，分成2份，分别供扫描电镜和透射电镜用.

扫描电镜与透射电镜检查 样品制备方法同前[5]，在 ISI-DS-130型扫描电镜与 JEM-2000 EX 透射电镜下观察. 每种样品各重复3次实验，每次每个样品制3—4个切片观察.

培养心肌细胞释放 LDH 测定[6] 每培养瓶为一样本，留取培养液0.1 ml，利用顺向反应，以乳酸为基质，在440 nm 波长下进行比色，检测在辅酶 I 存在时 LDH 使乳酸钠转化成丙酮酸的含量，以间接测定 LDH 活性.

RESULTS

Sal 对细胞搏动的影响 心肌细胞培养72 h，缺氧3 h，再给氧1 h 的各组细胞搏动频率见 Tab 1. 结果表明 Sal 组与对照组相比有明显增加心肌细胞搏动频率作用. 对照组为37±5 bpm，Sal 10和30 μg·ml^{-1}组各为71±8和78±9，P<0.01. 与 Ver 组 (78±5 bpm)相比作用相近. Sal 10与30 μg·ml^{-1}组对心肌细胞搏动频率的作用基本相似.

对细胞释放 LDH 含量的影响 Sal 组与对照组相比，心肌细胞释放 LDH 含量明显降低(对照组为1.51±0.22，Sal 10和30 μg·ml^{-1}组分别为0.99±0.20，0.89±0.19 μmol·s^{-1}·L^{-1}，P<0.01)与 Ver 组相比，无显著差异，Sal 10和30 μg·ml^{-1}组之间也无差异(Tab 1).

Tab 1. Effect of salidroside (Sal) on beat and lactate dehydrogenase (LDH) of cultured myocardial cells anoxia/reoxygenation injury. n=8, $\bar{x}\pm s$. ^cP<0.01 *vs* control, ^dP>0.05 *vs* verapamil.

Drug 0.1 ml μg·ml^{-1}	Beat rate/bpm	LDH/ μmol·s^{-1}·L^{-1}
Control	37±5	1.51±0.22
Sal 10	71±8^{cd}	0.99±0.20^{cd}
Sal 30	78±9^{cd}	0.89±0.19^{cd}
Verapamil 46.6	78±5^c	0.81±0.18^c

扫描电镜观察 对照组心肌细胞簇团块大多数分散解体成单个细胞，细胞多呈圆形，伪足变细或消失(Fig 1-A, Plate 2). Sal 10 μg·ml^{-1}组心肌细胞簇团块基本保持完整，外观正常，细胞多呈多角形，并有较多伪足，互相联接(Fig 1-B, Plate 2). Sal 30 μg·ml^{-1}组心肌细胞簇外观完整，细胞排列紧密，连成片状(Fig 1-C, Plate 2).

透射电镜观察 对照组心肌细胞以线粒体损害较为明显，表现为线粒体肿胀，嵴溶解缺失，基质中可见透光区，有的呈现"髓样变线粒体"或致密体，即缺氧样线粒体(anoxia-like mitochondria)；其次表现为肌浆网扩张；再者 Z 线，M 线不清晰(Fig 1-D, Plate 2). Sal 10 μg·ml^{-1}组心肌细胞结构基本正常，可见相邻心肌纤维以闰盘端端相连，闰盘呈阶梯状，并可见结构正常线粒体(Fig 1-E, Plate 2). Sal 30 μg·ml^{-1}组心肌细胞超微结构正常，肌原纤维排列整齐，可见 I 带，A 带 Z 线及 M 线，线粒体较丰富(Fig 1-F, Plate 2).

DISCUSSION

已知心肌细胞的缺血缺氧是导致细胞内酶释放的重要原因之一[7]，因此，心肌细胞酶释放可作为细胞完整性损伤的生化标志[8-9]. 本文观察到缺氧后再给氧心肌细胞不仅细胞搏动的生理功能受到影响，而且电镜观察到细胞膜完整性受到破坏，生化测定显示 LDH 释放量

增加提示心肌细胞膜断裂或缺损处具备了酶释放的超微病理形态基础[10].

本实验研究表明 Sal 对缺氧后再给氧损伤心肌细胞具有保护作用. 超微结构观察提示其主要作用机制在于维持心肌细胞膜的稳定性与完整性，因而使心肌细胞的搏动功能维持正常，避免细胞内 LDH 释放. 此外，还观察到 Sal 作用效果与钙离子通道阻滞剂 Ver 相似. 有报道心肌缺血再灌注损伤的重要发病环节是细胞内 Ca^{2+} 超载所致[11]，因此，Sal 是否具有钙离子通道阻滞样作用，有待研究.

REFERENCES

1 Chen QM, Jiang P. Electron microscope study of the protecting effect of Hongjingtian (*Rhodiola eccinea*) ethanol extract on myocardium of rats. *High Altit Med J* 1987; (1) : 20—2.

2 Jiang P, Chen QM, Wu GM, Gao XF, Huang LC, Kou XC. Effect of *Rhodiola coccinea* of Tibetan drug on hypoxic tolerance. *Qinghai Sports Sci Tech* 1985; (2) : 18—26.

3 Ming GQ, Xia GC, Zhang RG. Advances of Hongjingtian study. *Chin Trad Herb Drugs* 1988; **19** : 229—31.

4 Li LD, Gao HY, Zhang JM, Li YO, Zhang J. Influences of "Tu Mao Dong Qing № II" (3,4-dihydroxyacephenone) on the beating of cultured heart cell of neonatal rat. *J Trad Chin Med* 1980; **21** (6) : 68—70.

5 Ye YC, Chen QM, Hai P, Chai FL, Zhou SX. Effect of *d*-hydrastine on the ultrastructure of experimental *Echinococcus granulosus* cyst in mice. *Acta Pharmacol Sin* 1989; **10** : 185—7.

6 Xu SK, Lactate dehydrogenase determination. In: Shanghai Medical Laboratory, editor. *Laboratory methods in clinical biochemistry*. Shanghai: Shanghai Science and Technology Press, 1979 : 314—21.

7 Hearse DJ. Enzyme release during myocardical anoxia: a study of metabolic protection. *J Mol Cell Cardiol* 1975; **7** : 463—9.

8 Zwier JL, Rayburn BK, Flaherty JT, Weisfeldt ML. Recombinant superoxide dismutase reduces oxygen free radical concentrations in reperfused myocardium. *J Clin Invest* 1987; **80** : 1728—33.

9 Rao PS, Cohen MV, Mueller HS. Production of free radicals and lipid peroxides in early experimental myocardical ischemia. *J Mol Cell Cardiol* 1983; **15** : 713—6.

10 Ye YC, Chen QM, Long W, Ruan ZH. Comparative studies on myocardium structure and function in *Marmota himalayana* and rat. *Acta Theriol Sin* 1990; **10** : 197—202.

11 Pridjian AK, Levilsky S, Krukenkamp I, Silverman NA, Feinberg H. Intracellular sodium and calcium in the postischemic myocardium. *Ann Thorac Surg* 1987; **43** : 416—9.

426-429 (12)

BIBLID : ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 Sep; **14** (5) : 426—429

美西律对海马脑片突触功能缺氧损伤的保护作用[1]

张雪松，王天佑 (北京市神经外科研究所病理生理室，北京100050，中国)

R 972.2

Protection of mexiletine against hypoxic damage of synaptic function in hippocampal slices

ZHANG Xue-Song, WANG Tian-You
(*Department of Pathophysiology, Beijing Neurosurgical Institute, Beijing 100050, China*)

Received 1991-10-22 Accepted 1992-12-24
[1] Project supported by the National Natural Science Foundation of China, № 3890353.

ABSTRACT The evoked population spike (PS) and presynaptic fiber volley (PV) were recorded from the CA1 area in rat hippocampal slices. At the 3rd min of hypoxia, the amplitude of PS declined to 0.4±0.4 mV in control slices whereas to 1.2±1.2 mV or 1.5±0.4 mV in slices pretreated for 1 h with mexiletine (Mex) 10 or 100 μmol·L^{-1}, respectively. Thirty min after reoxygenation the amplitude of PS recovered to 11.1% of its original level in control slices, but to 47.6% or 65.0% in slices pretreated with Mex 10 or 100