

大黄素对小鼠腹腔巨噬细胞内

游离钙浓度的影响[△]

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摘要 用钙敏感的荧光指示剂Fura-2/Am定量分析大黄素对小鼠腹腔巨噬细胞内(Ca^{2+})_i的影响。结果显示:在巨噬细胞悬液中相继加入不同剂量的 CaCl_2 后, (Ca^{2+})_i明显增高($P < 0.01$)。巨噬细胞用适当剂量大黄素予处理10min, 使用上述不同剂量的 CaCl_2 后, (Ca^{2+})_i水平明显增高, 该实验进一步提示:大黄素可促进细胞内 Ca^{2+} 释放, 也可促进细胞外 Ca^{2+} 内流。而且大黄素对 $[\text{Ca}^{2+}]_i$ 的作用呈剂量依赖性。

关键词 大黄素 腹腔巨噬细胞 Ca^{2+} Fura-2/Am

大黄素(emodin, EMD)是从大黄中提取的有效成分, 文献报道^[1, 2]。EMD具有抗菌、抗癌和抑制免疫功能等作用。但最近本教研室观察到EMD可提高淋巴细胞内游离钙浓度^[3], 为进一步探讨EMD对免疫功能的影响, 本文应用钙荧光指示剂Fura-2/Am测定了EMD对小鼠腹腔巨噬细胞内游离钙浓度($[\text{Ca}^{2+}]_i$)的影响。

1 材料与方法

1.1 药品与试剂: Fura-2/Am; 中国科学院上海生理研究所提供。Eagle's MEM; 日本Nissui制药公司产品。EMD; Sigma公司产品。其它试剂均为市售分析纯。

1.2 动物: 小鼠雌雄兼用, 体重10~22g。

1.3 方法

1.3.1 小鼠腹腔巨噬细胞制备: 取小鼠4只断脊髓处死, 腹腔内分别注入2ml冰冷的Eagle's—MEM培养液, 轻揉腹部1min后打开腹腔, 分别取腹腔液2ml置一试管内, 用无 Ca^{2+} , Mg^{2+} 的Hank's液洗涤离心共3次(500r, 5min), 最后加MEM制成 $1 \sim 2 \times 10^7$ 细胞悬液备用。

1.3.2 Fura-2/Am负载及荧光测定: 取腹腔巨噬细胞悬液2ml, 加入Fura-2/Am(终浓度 $5 \mu\text{mol/L}$)和牛血清蛋白(0.1%), 在超级恒温水浴振荡器中孵育30min(37℃), 再用无 Ca^{2+} , Mg^{2+} 的Hank's液洗涤离心3次(500r, 5min)弃上清液, 最后将细胞悬浮在2ml无 Ca^{2+} , Mg^{2+} 的Hank's液中待测定。本实验采用岛津RF-510型(带恒温器的)荧光分光光度计, 将发射波长固定在490nm, 通过快速(4~6s)转换激发波长, 记录350/380nm的荧光比值变化, 由公式^[4]计算出细胞内游离钙浓度。其中 $k_d = 224 \text{ nmol/L}$, 在计算之前需减去未负载Fura-2/Am的细胞自发荧光:

$$[\text{Ca}^{2+}]_i = k_d \cdot (R - R_{\min}) \cdot (R_{\max} - R)^{-1} \cdot S$$

2 结果

2.1 CaCl_2 对腹腔巨噬细胞内 $[\text{Ca}^{2+}]_i$ 的影响: 在静息状态下, 小鼠腹腔内巨噬细胞在无 Ca^{2+} , Mg^{2+} 的Hank's液中, $[\text{Ca}^{2+}]_i$ 为 $198.56 \pm 44.82 \text{ nmol/L}$ ($n = 11$) 在细胞悬液中

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加入CaCl₂ (0.75, 1.75, 2.75mmol/L) 后, 腹腔巨噬细胞内[Ca²⁺]i均明显高于静息时水平 ($P<0.001$, 图1)。

2.2 EMD对CaCl₂引起腹腔巨噬细胞内[Ca²⁺]i增加的影响:腹腔巨噬细胞经EMD(4.6, 9.2, 8.5和37μmol/L)预先温育10 min后, 在静息状态下(0.5mmol/L EGTA存在), 高浓度EMD(37μmol/L)使腹腔巨噬细胞内[Ca²⁺]i较对照组明显增加 ($P<0.05$, 表1), 说明EMD可促进内钙释放。经适当剂量EMD作用的腹腔巨噬细胞悬液中加入上述等剂量的CaCl₂后, [Ca²⁺]i水平较对照组明显增高 ($P<0.05$ 和 $P<0.01$, 图1)。而且, 大黄素增加巨噬细胞内游离钙浓度的作用与剂量相关, 剂量增加, 效应增强(图2, 表)

表 静息状态下小鼠腹腔巨噬细胞内游离钙浓度 ($\bar{x} \pm SD$)

组别	例数	加EGTA前 [Ca ²⁺]i nmol/L	加EGTA后 (0.5mmol/L) [Ca ²⁺]i nmol/L
对照组 (与给药 组等量等pH水)	11	198.66 ± 44.82	26.60 ± 11.36
4.6	7	184.69 ± 43.26	24.91 ± 6.53
9.2	8	194.77 ± 35.64	23.54 ± 4.68
18.5 μmol/L	6	231.72 ± 37.56*	34.58 ± 8.81
37.0	7	290.02 ± 45.75***	39.46 ± 4.91*

注: 与对照组比较 * $P<0.5$ *** $P<0.01$

3 讨论

钙对细胞有极其重要的生理作用[5]。巨噬细胞在不同环境条件下可能发挥不同的免疫调节作用[6]。当细胞内钙增高到一定程度时, 钙作为激动剂使细胞产生效应, 低钙则可导致机体免疫功能不足。本实验结果表明, 适当浓度的大黄素不但可促进巨噬细胞内钙释放, 而且还可以促进外钙内流, 其效应与剂量相关。结果提示: 大黄素有促进机体免疫功能的作用。

参 考 文 献

- 1 陈琼华. 冶金医药情报, 1990, 7(3): 88
- 2 陈家坤, 等. 中草药, 1991, 22(12): 543
- 3 靳珠华, 等. 中草药, 1994, 25(8): 413
- 4 Crynkiewicz G, et al. J Bio Chem, 1985, 260(5): 3440
- 5 金有豫. 中国药学杂志, 1982, 27(1): 38
- 6 Nathan C F. J Clin Invest, 1987, 79, 319

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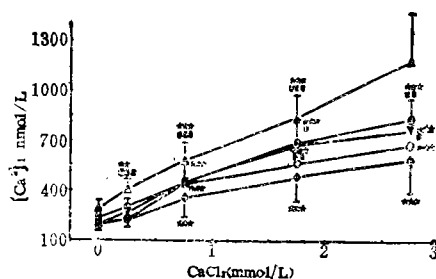


图1 EMD对CaCl₂诱导小鼠腹腔巨噬细胞内钙增加的影响

◇-对照组 (n=11) ○-EMD 4-6μmol/L (n=7)
▽-EMD9.2μmol/L (n=8) ◻-18.5μmol/L
(n=6) ▲EMD37μmol/L (n=7)
与给药前相比* $P<0.05$ ** $P<0.01$ *** $P<0.001$
与对照组相比* $P<0.05$ ** $P<0.01$ *** $P<0.001$

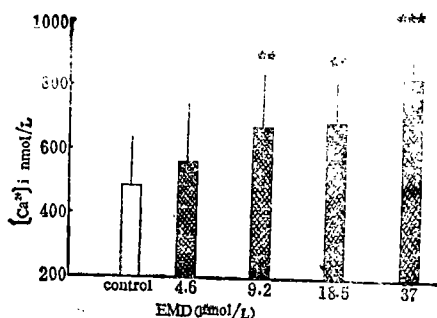


图2 EMD对CaCl₂ (1.75mmol/L) 小鼠腹腔巨噬细胞内钙增加的剂量关系

与对照组相比 ** $P<0.01$ *** $P<0.001$

Determination of Tanshenoside I in Taidangshen (*Codonopsis pilosula*) and Cultivated Ludangshen (*C. pilosula*) of Different Years Old

Dai Jing, Feng Li, Han Guiru et al

Amounts of tanshenoside I in cultivated Ludangshen of different years of cultivation history was determined by TLC densitometry in comparison with that in wildly grown Ludangshen and cultivated Taidangshen. Results showed that tanshenoside I in cultivated Ludangshen decreases with increased years of cultivation history while that in cultivated Taidangshen is slightly lower than that of Ludangshen of the same years of cultivation. Tanshenoside I in wild Taidangshen is also lower than that in cultivated Taidangshen.

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Study on Biological Effects of Songzike Acidic Polysaccharide

Hou Fangyu, Yu Qinghua, et al

The biological effects of Songzike acidic polysaccharide (ST90) were studied in vivo and vitro. It was found that ST90 had distinct antineoplastic, antibacterial and antiviral effects. It markedly inhibits proliferation of S180 solid tumor in mice and reduces the mortality of mice infected by *Salmonella typhimurium*, and shows a protective effect on FL cells infected with adv_1 , adv_2 , vsv and CB_4 .

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Protective Effect of Tea Polyphenol on Rat Myocardial Injury Induced by Isoproterenol

Tang Shengxing, Ye Ting, Zhao Zhengdong

Retreatment with tea polyphenol (TP) at a dose of 10mg/kg ip to rats five days before isoproterenol (ISO) challenge (1mg/kg sc, for two days), resulted in decreases of malondialdehyde concentration, creatine phosphokinase, lactic dehydrogenase (LDH) and LDH_1 activities, increased LDH_2/LDH_1 ratio and inhibited the extent of myocardial injury, similar to the action of propranol. At the same time TP decreased rat plasma renin activity. The results suggested that the mechanism by which TP protects heart from ISO-induced myocardial injury is due to its antioxygen free radical and inhibition of renin activities.

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Effects of Emodin on the Cytoplasmic free Ca^{2+} in Peritoneal Macrophages From Mice

Cui Rongfen, Lin Xiuzhen

Effect of Emodin (EMD) on free intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) in peritoneal macrophages of mice was measured with Ca^{2+} sensitive fluorescent indicator Fura-2/Am quantitatively. Results showed that in the resting, $[\text{Ca}^{2+}]_i$ level in the peritoneal macrophages was $198.56 \pm 44.82 \text{ nmol/L}$ ($n=11$) in Ca^{2+} free Hank's solution. After adding 0.75, 1.75 and 2.75 mmol/L CaCl_2 to macrophages suspension sequentially, the free $[\text{Ca}^{2+}]_i$ levels were obviously higher as compared with that of the resting level ($P < 0.01$). When the macrophages were pretreated with adequate doses of EMD for 10 min, in the resting or using the above doses of CaCl_2 , the $[\text{Ca}^{2+}]_i$ was significantly increased as compared with the control groups ($P < 0.01$). The present investigation indicated that EMD can not only promote the release of intracellular Ca^{2+} but also the influx of extracellular Ca^{2+} . Furthermore the effect of EMD on $[\text{Ca}^{2+}]_i$ is dose dependent.

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Studies on Resource Utilization of Chinese Drug

Dwarf Lilyturf (*Ophiopogon japonicus*)

Yu Boyang, Xu Guojun

"Maidong" (*Radix Ophiopogonis*), is a traditional Chinese drug. Chinese Pharmacopoeia (1990) recorded that the botanical origin of "Maidong" is *Ophiopogon japonicus* (L. f.) Ker-Gawl. (Liliaceae). A recent survey of drug resources in Zhejiang, Sichuan, Hubei, Hunan, Yunnan, Guizhou, Jiangsu, Anhui and Fujian Provinces showed that plants under the general name "Maidong" involve 26 species and varieties from Gen. *Ophiopogon* to Gen. *Liriope*. Among these, 16 species and varieties are used as "Maidong" in commerce, the most popular being *O. japonicus*, *L. spicata* var. *prolifera* and *L. muscari*. The steroid saponins and homeisoflavonoids contained in the tuberous roots of 16 species and varieties mentioned above were compared on the basis of HPTLC with 45 steroid saponins and 5 homeisoflavonoids as authentic samples. The results showed that the plants belonging to Gen. *Ophiopogon* contain either steroid saponins composed of ruscogenin or diosgenin, or homeisoflavonoids; the plants belonging to Gen. *Liriope* contain only steroid saponins composed of 25(S)-ruscogenin or yamogenin. It is easy to identify the varied species and varieties on TLC. The polysaccharide in the tuberous roots of the 16 species and varieties was determined by visible spectrophotometry. Results indicated that the polysaccharide content in varied species is very different, and is also dependent on locations, cultivated years and the grade of crude drug.

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Pharmacognostical Identification of Lawn Pennywort

(*Hydrocotyle sibthorpioides*)

Huang Chengyong, Luo Zeyun, et al

The botanical Origin, macroscopical and microscopical characteristics and physical chemical tests for the identification of Tianhusui (*Hydrocotyle sibthorpioides* Lam.) and Potongqian (*H. sibthorpioides* Lam. var. *batrachium* (Hance) Hand-Mazz.) were reported to provide a basis for the identification of the drug and revision of the drug standard.

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