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## 大黄素对肾小球系膜细胞 c-*myc* 原癌基因表达的影响

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**关键词** 大黄素；培养的细胞；肾小球系膜；原癌基因蛋白 c-*myc*

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**目的**：观察大黄素对肾小素系膜细胞(MC) c-*myc* mRNA 表达的影响，探讨大黄素抑制 MC 生长的分子机理. **方法**：网筛法分离大鼠肾小球，培养肾小球 MC. AGPC 一步法提取细胞总 RNA，c-*myc* mRNA 水平用斑点杂交法测定，以显影斑点最大的 RNA 稀释度表示 mRNA 水平. **结果**：生长相对静止状态的 MC 有低水平 c-*myc* mRNA 表达，而细菌脂多糖(LPS)显著增高 MC c-*myc* mRNA 表达. 于 2.5 h 达最高峰，并持续 6 h. LPS 诱导的 MC 高表达 c-*myc* mRNA 可被大黄素(25 mg · $L^{-1}$)所抑制. **结论**：大黄素对 MC c-*myc* mRNA 表达的抑制效应与其抑制 MC 的生长相关.

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# Effects of egtazic acid and calcimycin on synthesis of DNA and collagen in cultured human lung fibroblasts

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**KEY WORDS** egtazic acid; calcimycin; DNA; collagen; fibroblasts; cultured cells

**AIM**: To study the effects of egtazic acid (EA) and calcimycin (Cal) on the synthesis of DNA and collagen in cultured human lung fibroblasts (HLF). **METHODS**: The synthesis of DNA and collagen was determined by measuring the incorporation of [$^3$H]TdR and [$^3$H]proline of HLF respectively. **RESULTS**: The collagen synthesis increased markedly 24 h after exposure to both EA (0.05－4 mmol · $L^{-1}$) and Cal (0.25－20 μmol · $L^{-1}$), and that there was no obvious change in DNA synthesis. After 36－48-h exposure, both DNA and collagen syntheses decreased in the groups of EA (1, 2, and 4 mmol · $L^{-1}$); the DNA synthesis was also suppressed in Cal groups in a concentration-dependent manner, whereas collagen synthesis decreased only in Cal (10 and 20 μmol · $L^{-1}$). **CONCLUSION**: Extracellular $Ca^{2+}$ influx into fibroblasts increased collagen production. However, the DNA synthesis was suppressed when the cytosolic $Ca^{2+}$ was too high or too low.

$Ca^{2+}$ plays an important role in modulating cell proliferation and functional activities[1,2]. Most studies focused on the effects of $Ca^{2+}$ on nerve cells, myocardium cells, smooth muscle cells, and tumor cells[3－5]. Few articles dealt with the influences of $Ca^{2+}$ on DNA and collagen synthesis of fibroblasts. Organ fibroses, such as pneumonocirrhosis and hepatocirrhosis, result from an overproliferation of fibroblasts and excessive collagen synthesis. To determine the effects of $Ca^{2+}$ influx on the DNA and collagen syn-

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thesis of fibroblasts and investivgate the potentiality of treating organ fibroses with $Ca^{2+}$ channel blockers, we observed the influences of egtazic acid (EA) and calcimycin (Cal, a calcium ionophore) on the incorporation rate of [$^3$H]TdR and [$^3$H]proline in cultured human lung fibroblasts (HLF).

## MATERIALS AND METHODS

**HLF** HLF was provided by Shanghai Institute of Cell Biology, Chinese Academy of Sciences.

**Reagents** EA was the product of Huzhou Biochemical Factory. Cal (Sigma). RPMI-1640 medium (Gibco). [$^3$H]TdR and [$^3$H]proline were purchased from Shanghai Institute of Nuclear Research, Chinese Academy of Sciences and Chinese Academy of Atomic Energy Science, respectively. Scintillation liquid was xylene containing 0.5 % PPO and 0.005 % POPOP. Other reagents are all of AR.

**Measurement of DNA and collagen synthesis** HLF was suspended in RPMI-1640 medium ($1.2 \times 10^5$ cells · $L^{-1}$) supplemented with 10 % calf serum containing penicillin 100 kU · $L^{-1}$ and streptomycin 100 mg · $L^{-1}$, and were plated in 48-well cell culture cluster dishes. Cells were grown at 37 C in a humidified 5 % $CO_2$+95 % air. After 24-h incubation, EA or Cal was added, and for the untreated controls an equal amount of drug-solvent (RPMI-1640 containing 1 % $NaHCO_3$, 7.4 % ethanol) was added. DNA and collagen synthesis was assessed by measuring the incorporation rates of [$^3$H] TdR and [$^3$H]proline. The period of exposure to [$^3$H]TdR or [$^3$H] proline was 24 h. The final concentrations of [$^3$H]TdR and [$^3$H]proline were 37 and 296 MBq · $L^{-1}$, respectively. At the termination of culture, the cells were treated with 0.25 % trypsin and harvested onto glass fiber filters, which were washed with trichloroacetic acid 0.6 mol · $L^{-1}$ after rinsing with 0.9 % NaCl. The precipitates were dehydrated and decolorized with ethanol, and stoved at 80 ℃. The radioactivities (dpm) were counted in a liquid scintillation counter (YSJ 80). Data were expressed as $\bar{x} \pm s$ ($n$=3 wells).

Statistical significance was assessed by ANOVA (Dunnett analysis).

## RESULTS

**Cell morphology** Under light microscope, HLF grew well in all groups of Cal (0.25 — 20 μmol · $L^{-1}$). In the groups of EA (0.05 and 0.1 mmol · $L^{-1}$), cells showed no obvious change. After 12-h exposure to EA, the cells shrank at 2 or 4 mmol · $L^{-1}$; the cell distribution appeared to be slightly sparse but there was no obvious change in shape at 1 mmol · $L^{-1}$. After 24 h or longer of exposure to EA, the cells became round in all groups >1 mmol · $L^{-1}$.

**Effects of EA and Cal on DNA synthesis** The incorporation of [$^3$H]TdR was not remarkably affected in Cal 0.25—20 μmol · $L^{-1}$ after 24-h exposure. But, it was suppressed in a concentration-dependent manner after 36—48 h.

DNA synthesis was not influenced after addition of EA 0.05—4 mmol · $L^{-1}$ for 24 h. After 36 — 48 h, DNA synthesis was concentration-dependently suppressed in 1—4 mmol · $L^{-1}$, and the suppression rate increased along with 36 — 48 h.

**Effects of EA and Cal on collagen synthesis** Collagen synthesis increased in all groups of Cal and EA after 24-h exposure. But after 36—48-h exposure, collagen synthesis decreased remarkably in Cal (10 and 20 μmol · $L^{-1}$) and EA (1, 2, and 4 mmol · $L^{-1}$) (Tab 1).

## DISCUSSION

As a $Ca^{2+}$ carrier, Cal can elevate the cytosolic $Ca^{2+}$ concentration by transporting $Ca^{2+}$ from extracellular fluid into the cytosol[3]. The incorporation rate of [$^3$H]proline increased markedly and the DNA synthesis was not influenced after 24-h exposure to Cal. These showed that an elevated cytosolic $Ca^{2+}$ concentration promoted collagen synthesis of HLF, and here $Ca^{2+}$ influx was important. As to the collagen synthesis increased after 24-h exposure to EA, we presume that the chelation of $Ca^{2+}$ in the medium can prevent the $Ca^{2+}$ influx into the cells, resulting in a compensatory release of $Ca^{2+}$ from intracellular stores into the cytosol. But we did not measure the cytosolic $Ca^{2+}$ to demonstrate it.

Both Cal and EA suppressed the DNA synthesis of HLF after 36—48-h exposure, we deem that $Ca^{2+}$ probably possesses a double-direction regulation on DNA synthesis, viz DNA synthesis requires certain amount of $Ca^{2+}$, but when the cytosolic $Ca^{2+}$ concentration is beyond the upper-limit, DNA synthesis will be suppressed on the contrary. This was in consensus with the results of literature[2,4].

In conclusion, the elevated cytosolic $Ca^{2+}$ promoted collagen synthesis of HLF, in which $Ca^{2+}$ influx played a significant role. The DNA

**Tab 1. Effect of calcimycin and egtazic acid on syntheses of DNA and collagen in cultured human lung fibroblasts.** $n=3$ wells, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

| | | DNA | | | Collagen | | |
|---|---|---|---|---|---|---|---|
| | | 24 h | 36 h | 48 h | 24 h | 36 h | 48 h |
| Calcimycin | 0 | 4 514±38 | 6 326±15 | 6 102±75 | 1 279±129 | 3 175±434 | 3 046±251 |
| (μmol·L$^{-1}$) | 0.25 | 4 544±130$^a$ | 4 686±120$^c$ | 4 461±47$^c$ | 3 196±222$^c$ | 3 146±77$^a$ | 2 744±259$^a$ |
| | 2.50 | 4 087±22$^a$ | 3 592±63$^c$ | 3 263±20$^c$ | 3 507±665$^c$ | 2 734±157$^a$ | 2 796±210$^a$ |
| | 5.0 | 3 988±72$^a$ | 3 916±12$^c$ | 2 487±80$^c$ | 2 919±237$^c$ | 3 128±15$^a$ | 2 814±194$^a$ |
| | 10.0 | 4 150±109$^a$ | 3 116±35$^c$ | 2 014±72$^c$ | 2 979±227$^c$ | 2 702±274$^b$ | 2 438±339$^b$ |
| | 20.0 | 3 180±59$^a$ | 2 722±205$^c$ | 1 827±152$^c$ | 3 075±224$^c$ | 2 669±241$^b$ | 2 502±217$^b$ |
| Egtazic acid | 0 | 6 710±1 239 | 6 221±1251 | 5 858±262 | 1 595±302 | 3 199±122 | 2 587±261 |
| (mmol·L$^{-1}$) | 0.05 | 4 098±603$^a$ | 6 436±332$^a$ | 4 529±1 084$^a$ | 2 697±331$^c$ | 3 009±391$^a$ | 2 473±209$^a$ |
| | 0.10 | 5 003±1 150$^a$ | 4 783±1 171$^a$ | 5 306±409$^a$ | 2 198±122$^c$ | 3 447±150$^a$ | 2 547±464$^a$ |
| | 1.0 | 4 456±399$^a$ | 2 958±454$^c$ | 2 421±70$^c$ | 2 353±182$^c$ | 1 553±404$^b$ | 1 558±122$^b$ |
| | 2.0 | 4 033±491$^a$ | 2 898±483$^c$ | 2 329±232$^c$ | 2 320±524$^c$ | 1 456±134$^b$ | 1 398±5$^b$ |
| | 4.0 | 3 779±933$^a$ | 2 669±82$^c$ | 727±25$^c$ | 2 448±172$^c$ | 1 269±55$^b$ | 1 603±397$^b$ |

synthesis was suppressed when the cytosolic $Ca^{2+}$ concentration was too high or too low. The results suggest that $Ca^{2+}$ antagonists may be a group of potential useful drugs for antifibrosis.

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## 依他酸和卡西霉素对人胚肺成纤维细胞脱氧核糖核酸和胶原合成的影响

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**关键词** 依他酸；卡西霉素；脱氧核糖核酸；胶原；成纤维细胞；培养的细胞

**目的**：研究依他酸(EA)和卡西霉素(Cal)对人胚肺成纤维细胞脱氧核糖核酸和胶原合成的影响. **方法**：采用[$^3$H]TdR与[$^3$H]脯氨酸掺入分别测定脱氧核糖核酸和胶原合成. **结果**：给药24 h后，EA 0.05－4 mmol·L$^{-1}$和Cal 0.25－20 μmol·L$^{-1}$组胶原合成均显著增加，脱氧核糖核酸合成无明显改变. 给药36和48 h，EA 1－4 mmol·L$^{-1}$组胶原与脱氧核糖核酸合成降低；Cal 0.25－20 μmol·L$^{-1}$组脱氧核糖核酸合成亦下降，胶原合成仅10和20 μmol·L$^{-1}$组降低. **结论**：细胞外液$Ca^{2+}$内流促进胶原合成，细胞内$Ca^{2+}$浓度过高或过低均抑制脱氧核糖核酸合成.