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摘要 I-65 30-100 $\mu\text{mol}\cdot\text{L}^{-1}$ 可缩短豚鼠单个离体心室肌细胞的 APD₂₀。采用单微电极电压钳方法, 发现上述浓度的 I-65可明显抑制心室肌细胞的 I_{Ca}, 但不影

响其阈电压和反转电压。I-65对 I_{Ca}的抑制呈使用依赖性。提示: I-65可阻断钙通道, 并可能作用于钙通道的失活态。

关键词 碘化合物; I-65; 动作电位; 心肌; 钙通道阻滞剂; 膜电位

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Effect of *Panax notoginseng* saponins on increased proliferation of cultured aortic smooth muscle cells stimulated by hypercholesterolemic serum

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ABSTRACT *Panax notoginseng* saponins (PNS) was extracted from a Chinese herb medicine. After preparation of cultured aortic smooth muscle cell (SMC) from primary aortic explants, the cytotoxicity of hypercholesterolemic serum (HCS) for cultured cells was determined by trypan blue exclusion test, and [³H]thymidine incorporation and cell numbers were counted at the same time. The results showed that HCS (0.5 mg cholesterol·ml⁻¹) increased the incorporation of [³H]thymidine into cultured cells (3722 ± 440 vs 1655 ± 288 dpm/μg cell protein, P < 0.01), stimulated the proliferation of SMC [(6.5 ± 1.5) × 10⁶ vs (4.3 ± 1.2) × 10⁵ cells/plate, P < 0.01], and that high concentration HCS (final cholesterol concentration 2 mg·ml⁻¹) was cytotoxic to the cultured cells. PNS (100 and 400 μg·ml⁻¹) decreased the incorporation of [³H]thymidine into SMC in culture with or without HCS (1292 ± 260 and 982 ± 314 or 4111 ± 886 and 2361 ± 751 dpm/μg cell protein), and inhibited the proliferation of the cultured cells [(3.3 ± 0.7) × 10⁵ and (2.9 ± 0.7) × 10⁵ or (4.7 ± 1.4) × 10⁵ and (4.1 ± 1.2) × 10⁵ cells/plate). We conclude that PNS can inhibit the proliferation of aortic SMC stimulated by HCS. These results also suggest that HCS may play an atherogenic role in the arterial wall and that PNS may prevent atherosclerosis and inhibit progression of

the atherosclerotic lesions by interfering with the proliferation of arterial SMC.

KEY WORDS ginseng; saponins; cultured cells; thoracic aorta; vascular smooth muscle; cell count; hypercholesterolemia; thymidine

Hypercholesterolemic serum (HCS) is known to cause proliferation and necrosis in cultured smooth muscle cells (SMC)⁽¹⁾. In this study, we used HCS to stimulate the proliferation of cultured SMC, and studied the effects of *Panax notoginseng* saponins (PNS) on the HCS-induced DNA synthesis and cell growth of cultured aortic SMC. As PNS can retard the progress of atherosclerosis in rabbits⁽²⁾, we raise the possibility that PNS can inhibit the development and progress of atherosclerotic lesions by interfering with the proliferation of arterial SMC.

MATERIALS AND METHODS

PNS, which has seven stains in thin-layer chromatographic identification⁽³⁾, was purchased from Wuzhou Third Pharmaceutical Factory. Trypsin and Medium 199 (M199) were obtained from Grand Biological Co, Grand Island NY, USA. [³H]Thymidine

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was obtained from China Institute of Atomic Energy (Beijing). Rabbits were provided by Laboratory Animal Center of SUN Yat-Sen University of Medical Sciences.

Culture of aortic SMC Cultured aortic SMC were prepared according to Pearson⁽⁴⁾. The primary explants from the rabbit thoracic aorta were transferred. To minimize the detachment of explants, the culture flask was placed upright in the incubator. After 18–24 h it was placed horizontally allowing the medium to cover the explants. The primary SMC were trypsinized and subcultured after the culture reached confluency. Cells in passages 5–7 were used for the experiment.

HCS preparation⁽⁴⁾ HCS was obtained by feeding the rabbits with an atherogenic diet containing 0.5% cholesterol for at least 10 wk. Blood serum was inactivated by heating at 56°C for 30 min and sterilized by ultrafiltration (0.2 μm). The concentrations of cholesterol, triglycerides, and HDL-cholesterol in HCS were 18.35, 4.60, and 49.0 g·L⁻¹, respectively.

Cell injury test The cytotoxicity of hypercholesterolemic serum for the cultured cells was determined by trypan blue exclusion test⁽⁶⁾.

DNA synthesis and growth of cells A cultured cell suspension (0.5 × 10⁵ cells·ml⁻¹) was prepared on d 1 using M199 with 10% newborn bovine serum (NBS). One ml of this suspension was distributed to each well of a 24-well plate and incubated for 48 h. Different experimental media were added to each group of 8 wells. The media were replaced 48 h after the subculture with the experimental media and [³H] thymidine was added simultaneously to a final radioactivity of 37 kBq·ml⁻¹. After 24 h, the media were removed, and the cultured cells were rinsed thrice with cold buffer solution (tris-HCl 50 mmol·L⁻¹, pH 7.4). The cells were then dissolved in 1 ml of NaOH 0.1 mol·L⁻¹, and 0.5 ml of this solution were mixed with 5 ml of scintillator. The intracellular radioactivity of [³H] was measured with a liquid-scintillation counter after standing overnight at 23°C. The residual solution was prepared for the determination of intracellular protein concentration⁽⁶⁾. DNA synthesis as determined by intracellular radioactivity in the culture was expressed as dpm/μg cell protein.

Cell number was counted by blood cell counting plate after 7 d incubation.

Experimental procedure The media with HCS (final cholesterol concentrations were 0.5, 1, and 2 mg·kg⁻¹) were used in the cultures. One concentration of HCS (final cholesterol concentration 0.5 mg·ml⁻¹) was used in the following experiment: (1) control medium, (2) medium with HCS, (3) medium with HCS + PNS (final concentration 100 μg·ml⁻¹), and (4) medium with HCS + PNS (final concentration 400 μg·ml⁻¹). DNA synthesis during 24 h and total amount in 7 d culture were measured. The significance of the difference was evaluated by *t* test.

RESULTS

Effects of HCS on cultured aortic SMC

The proportion of dead cells in SMC was determined by the trypan blue exclusion test. Three concentrations of HCS in the media were tested (final cholesterol concentrations were 0.5, 1, and 2 mg·ml⁻¹). Two HCS concentrations (cholesterol 1 and 2 mg·ml⁻¹) increased the proportion of dead cells *vs* the control (20% ± 3% and 28% ± 4% *vs* 10% ± 4%, both *P* < 0.01), low concentration of HCS had no significant effect on the percent of dead cells, but increased the incorporation of [³H]thymidine *vs* the control (3722 ± 440 *vs* 1655 ± 288 dpm/μg cell protein, *P* < 0.01). After incubation for 7 d with 3 concentrations of HCS, only low concentration (cholesterol 0.5 mg·ml⁻¹) of HCS increased the total number of cultured cells *vs* the control [(6.5 ± 1.5) × 10⁵ cells/plate *vs* (4.3 ± 1.2) × 10⁵ cells/plate, *P* < 0.01], while high concentration of cholesterol caused a decrease in the number of cultured cells [(2.5 ± 0.7) × 10⁵ cells/plate].

Effects of PNS on proliferation of cultured SMS PNS at the final concentrations of 100 and 400 μg·ml⁻¹ decreased the incorporation of [³H]thymidine *vs* the control (1292 ± 260 and 982 ± 314 *vs* 1774 ± 442 dpm/μg cell protein, *P* < 0.05, *P* < 0.01), and inhibited the increased [³H]thymidine incorporation by HCS (4111 ± 886 and 2361 ± 752 *vs* 2828 ± 505

dpm/ μ g cell protein, both $P < 0.01$). After incubation for 7 d, PNS decreased significantly the total number of cultured SMC vs the control $[(3.3 \pm 0.7) \times 10^5$ and $(2.9 \pm 0.7) \times 10^5$ vs $(4.5 \pm 1.0) \times 10^5$ cell/plate, $P < 0.05$ and $P < 0.01$], and also inhibited the HCS-increased growth of SMC $[(4.7 \pm 1.4) \times 10^5$, $(4.1 \pm 1.2) \times 10^5$ vs $(7.2 \pm 1.7) \times 10^5$ cells/plate, both $P < 0.01$].

DISCUSSION

We found that HCS could stimulate the proliferation of SMC *in vitro*, and that PNS could inhibit the incorporation of [³H]thymidine and growth of cultured SMC increased by HCS. In our previous studies, PNS was found to inhibit the necrosis of cultured vascular SMC by HCS and to reduce the ⁴⁵Ca uptake in those cultured cells (submitted for publication). We assumed that these effects had something to do with the calcium antagonism of PNS, because $[Ca^{2+}]_i$ influenced a number of important cellular functions involved in atherogenesis⁽⁷⁻⁹⁾. The exact anti-atherogenic mechanism of PNS and other calcium antagonists were not clear. Although PNS could inhibit the progress of experimental atherosclerosis by modulating the unbalance of PGI₂/TXB₂ in atherosclerotic rabbits⁽²⁾, we conclude that PNS may also prohibit the development of atherosclerosis by depressing the proliferation of aortic SMC.

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314-316 (8)

三七皂甙对高脂血清所致的培养主动脉平滑肌细胞增殖的作用

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摘要 本文采用体外培养血管平滑肌细胞(SMCs)的方法, 研究三七皂甙(PNS)对 SMCs 的保护作用. 结果表明, PNS 抑制 [³H]thymidine 掺入及细胞增殖. 低浓度高脂血清(HCS)促进 [³H]thymidine 掺入及细胞增殖. PNS (100 μ g·ml⁻¹, 400 μ g·ml⁻¹)能显著抑制 HCS 对 SMCs 的作用. 结果提示了 PNS 对于动脉粥样硬化的发生及发展可能具有一定的影响.

关键词 人参, 皂甙类, 培养的细胞, 胸主动脉, 血管平滑肌, 细胞计数, 血脂胆固醇过多症, 胸腺嘧啶核甙