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Effect of captopril on proliferation of aortic smooth muscle cells

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KEY WORDS captopril; thoracic aorta; vascular smooth muscle; cultured cells; hyperlipidemia; thymidine; 6-ketoprostaglandin F1 alpha; cyclic AMP; malondialdehyde; plasminogen inactivators

AIM: To study the effect of captopril (Cap) on proliferation of smooth muscle cells (SMC). **MATHODS**: Proliferation of cultured porcine aortic SMC was promoted by hyperlipidemic sera (HLS). **RESULTS**: Cap (0.2 mg·L^{-1}) remarkably reduced [^{3}H]thymidine ([^{3}H]TdR) incorporation, inhibited DNA synthesis and proliferation of SMC, and lessened morphological changes caused by HLS. After administration of Cap, malondialdehyde (MDA) content and plasminogen activator inhibitor (PAI) activity were reduced, 6-keto-$PGF_{1\alpha}$ and cAMP contents were obviously increased ($P<0.05-0.01$). **CONCLUSION**: Cap inhibited the proliferation of SMC, associated with MDA, PAI, 6-keto-$PGF_{1\alpha}$ and cAMP.

Percutaneous transluminal coronary angioplasty (PTCA) was one of the progresses in the treatment of coronary disease. But restenosis rate of arteries was about 35 % within 6 months after the operation[1]. Smooth muscle cell (SMC) proliferation plays a key role in leading vascular restenosis[2]. The researches in inhibiting proliferation of vascular SMC are in progress[3].

Angiotensin Ⅱ gives promoting to the proliferation of SMC. Therefore the effect of angiotensin-coverting enzyme (ACE) inhibitors on inhibiting the proliferation of SMC has been put in an important position[4,5]. Captopril (Cap) is one of the important ACE inhibitors. So its effect on proliferation of SMC and its influence on 6-keto-$PGF_{1\alpha}$, cAMP or malondialdehyde (MDA) content and plasminogen activator inhibitor (PAI) activity were studied.

MATERIALS AND METHODS

Culture of porcine aortic SMC Primary culture of porcine thoracic aortic (Wuhan Meat-Packing Plant) SMC were obtained by the explant technique. Two third media of aorta was cut into 0.5 – 1 mm^2 fragments and installed separately in culture vial containing 10 % fetal calf serum and medium M 199 (Gibco), then kept in incubator. The cells were subcultured at 7 – 10 d. Hyperlipidemic sera (HLS) was collected from 5 Japanese rabbits ♂, 3-month old, weighing 2.5 ± s 0.5 kg (Animal Center of Tongji Medical University) fed with 2 % cholesterol and 2 % pork fat forage for 8 wk. The total cholesterol of HLS was 39.78 mmol·L^{-1}.

Experiment groups and collection of sample The stable SMC that were growing between 2 – 3 generations were made into cell suspension and inoculated on cultural plates of 24 cavities (0.5×10^5). They were divided into 3 groups: a) control group: cultured with 10 % fetal calf serum and cultural medium M 199 b) HLS group: cultured with 10 % HLS and cultural medium M 199. c) Cap group: cultured also with 10 % HLS and cultural medium M 199, but containing Cap (made in Pharmaceutical Factory of Wuhan 4th Hospital) 0.2 mg·L^{-1}. All samples of 3 groups were incubated for 24 h. After the above interventions, the culture medium of each group was collected for measurement of 6-keto-$PGF_{1\alpha}$ (metabolism product of PGI2), MDA (metabolism product LPO) content, and PAI activity. The scraped SMC were used for measurement of cAMP content, and the SMC growth on slide were used for examination of light and electron microscopes.

Measurement methods Conditioned culture medium was centrifuged at 1000 × g for 10 min. The supernatant was collected to measure 6-keto-$PGF_{1\alpha}$ content with RIA method (kit, Suzhou Medical College), PAI activity with chromogenic substrates method (kit, Shanghai Medical University) and MDA content with improved TBA fluorescence method in which the standard product was 1,1,3,3-oxy-propane (FLUKA) cAMP content in SMC was determined by RIA method (kit, Shanghai University of Traditional Chinese Medicine and Pharmacology) after cells were diluted to 0.5×10^6·L^{-1} in culture vails, and were kept at 100 ℃ boiling water for 5 min Radioactivity count method was used to determine the [^{3}H] TdR (Shanghai Institute of Nuclear Research) incorportion into SMC

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The structure of alive cells was directly observed and taken pictures under phase contrast microscope. The cells after fix, dehydrate, embedding, section, and dyeing were observed and photographed undertransmissional electron microscopy.

All data were presented as $\bar{x} \pm s$. Statistical analyses between groups were determined using t test.

RESULTS

Morphology Under light microscope, all cells of 3 groups were arranged parallel to each other as parallelogram. Some cells were one on top of another like "peak and valley." Under electron microscope, rough surfaced endoplasmic reticulums and mitochontria were obviously increased, many fat drops could be seen, some pinocytotic vesicles were under the cytomembrane and microfilament were lesser in HLS group (Fig 1A). While in Cap group, rough surfaced endoplasmic reticulums and

Fig 1. In captopril group rough endoplasmic reticulums, mitochontria, and fat drops (see arrows) were less than those in hyperlipidemic sera group. ×3800. A: Hyperlipidemic sera group. B: Captopril group.

mitochontria were lesser than in HLS group and microfilaments were situated round the cells (Fig 1B).

Index in conditioned culture medium and cells

HLS group had a significantly higher [^{3}H]TdR data, MDA content, PAI activity, and a lower 6-keto-$PGF_{1\alpha}$, cAMP content than control group (all $P<0.01$). After administrations of Cap, [^{3}H]TdR data, MDA content, and PAI activity were significantly reduced, while 6-keto-$PGF_{1\alpha}$ and cAMP contents were obviously increased ($P<0.05$ or 0.01) (Tab 1).

DISCUSSION

Hyperlipidemia which is an important dangerous factor for coronary diseases can promote the proliferation of SMC[6]. In our experiment, HLS group had a significantly higher [^{3}H] TdR incorporation data than control group with obvious morphological changes. These meant that HLS could promote DNA synthesis and cell proliferation. However, [^{3}H]TdR incorporation data in Cap group were lower than in HLS group. Rough surfaced endoplasmic reticulums, mitochontria, and fat drops in Cap group were not as rich as in HLS group under light and electron microscopy. These results indicated that there were no obvious DNA synthesis and proliferation of SMC in Cap group. It suggested that Cap could inhibit the proliferation of SMC caused by HLS.

The remarkable increase of MDA in HLS group showed that HLS might accelerate cell proliferation through resulting in the lipid peroxidation of unsaturated fatty acid on membrane of cells[7]. The lower MDA in Cap group showed that Cap reduced the lipid peroxidation and inhibited SMC proliferation in the meantime.

Normal SMC can synthesize PGI2 using arachidenic acid (AA) in phospholipid of membrane and in external environment[8]. Our experiment proved that HLS reduce 6-keto-$PGF_{1\alpha}$, but Cap increased 6-keto-$PGF_{1\alpha}$ that can stimulate adenosine cyclase to enhance cAMP which can directly restrain the proliferation of SMC.

In addition, after using Cap, PAI activity was significantly decreased, which might result from the direct effect of Cap inhibiting the synthesis of PAI or decreasing the number and function of SMC. PAI is a special inhibitor to plasminogen activator (PA) which is an important physiological activator in fibrinolytic systm If PAI activity increases, thrombosis may occur. The formed thrombus and released various growth factors can stimulate the proliferation of SMC[9]. So, Cap may also indirectly inhibit the proliferation of SMC through decreasing PAI activity. It has been discovered that the increase of cAMP content in cells can transmit messages in cells and affect PA, PAI activities. Therefore, the change of PAI activity might be related to the increase of cAMP[10].

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Tab 1. The amount of [^{3}H]TdR corporation and the level of 6-keto-$PGF_{1\alpha}$, cAMP, MDA, and PAI in 3 groups. $n=10$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control; $^eP<0.05$, $^fP<0.01$ *vs* HLS.

Groups	[^{3}H]TdR/ dpm	6-keto-$PGF_{1\alpha}$/ ng·L^{-1}	cAMP/ nmol·L^{-1}	MDA/ μmol·L^{-1}	PAI AU·L^{-1}
Control	723 ± 133	51 ± 11	3.77 ± 0.93	0.17 ± 0.06	510 ± 70
HLS	877 ± 120^c	37 ± 10^c	2.67 ± 0.72^c	0.26 ± 0.06^c	660 ± 100^c
Cap/0.2 mg·L^{-1}	721 ± 126^f	51 ± 10^f	3.40 ± 0.78^e	0.16 ± 0.08^f	580 ± 60^{be}

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卡托普利对主动脉平滑肌细胞增殖的影响

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关键词 卡托普利；胸主动脉；血管平滑肌；培养的细胞；高脂血清；胸苷；6-酮前列腺素 $F_{1\alpha}$；环腺苷一磷酸；丙二醛；血纤维蛋白溶酶原失活剂

目的：观察卡托普利（Cap）对平滑肌细胞（SMC）增殖的作用及其作用机制。**方法**：用高脂血清（HLS）造成体外培养猪主动脉 SMC 增殖 **结果**：HLS 培养促进 SMC 增殖，加 Cap（0.2 mg·L^{-1}）后拮抗 SMC 增殖，使丙二醛（MDA）含量，纤溶酶原激活物抑制物（PAI）活性明显减少，6-酮-前列腺素 F1α（6-keto-$PGF_{1\alpha}$）及环腺苷一磷酸（cAMP）含量明显增加（$P<0.05$－0.01） **结论**：Cap 抑制 SMC 增殖，与 MDA，6-keto-$PGF_{1\alpha}$，cAMP 及 PAI 相关

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