APS 存在的条件下诱生的 DCs 刺激自体和异体 T 淋巴细胞的能力显著高于无 APS 存在条件下诱生的 DCs, 再次表明前者诱生的 DCs 具有更为成熟的免疫表型。

APS 是当归中的重要成分, 已有实验表明 APS 能从 mRNA 水平与蛋白质水平调控 GM-CSF 的表达, APS 可能通过直接或间接途径促进淋巴细胞、造血微环境中的基质细胞、巨噬细胞合成和分泌 GM-CSF 或其他造血生长因子。研究表明, APS 能在体外促进小鼠脾淋巴细胞 IFN-γ的分泌和提高 IFN-γ的生物活性[15]。而 IFN-γ能促进 DCs 的成熟[16]。在本实验中, 推测 APS 对 DCs 的刺激作用机制可能为: (1) 增强骨髓细胞 GM-CSF 自分泌作用。(2) 诱导骨髓细胞 IFN-γ分泌增加。

目前, DCs 用于肿瘤免疫治疗存在两个方面难题, 一方面, 尚未找到能大量扩增 DCs 的因子。另一方面, 扩增到一定时间和一定数量规模的 DCs 是否仍能保留其良好的抗原呈递功能。本研究结果表明 APS 能提高 DCs 的表面共刺激分子的表达, 促进其成熟并提高存活率, 延长 DCs 的生存时间。这预示 APS 与其他细胞因子联合诱导 CML-DCs, 对慢性粒细胞白血病的治疗可能有良好的前景。

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Effect of polydatin on endothelial function in aorta vascular strips of healthy rabbits treated with ADMA

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Abstract: Object To study the effect of polydatin (PD) and asymmetric dimethylarginine (ADMA)

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on the endothelial function in aorta vascular strips of healthy rabbits and the interaction between PD and ADMA. **Methods** Dose-response curves of phenylephrine (PE) on the aortic strips with or without ADMA and/or PD. E_{max} and K_d from PE were obtained and compared. **Results** Normal aortic strips could not respond to ADMA. Pretreatment with either PD or ADMA had no effect on the contractive response of aortic strips to PE. But PD could significantly weaken the contractive response of aortic strips pretreated with ADMA caused by PE in a dose-dependent manner, increase K_d and decrease E_{max} of PE. **Conclusion** Either ADMA or PD alone does not influence the normal aortic strip contractive functions, the PE affinity to α-receptor, and E_{max}. But PD could noncompetitively antagonize the contractive reaction of aortic strips to PE in the presence of ADMA.

Key words: polydatin (PD); asymmetric dimethylarginine (ADMA); nitric oxide synthase; phenylephrine (PE)

虎杖苷对 ADMA 作用的正常兔主动脉血管条内皮功能的影响

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摘 要: **目的** 研究虎杖苷 (PD) 与非对称性二甲基精氨酸 (ADMA) 对正常兔主动脉血管条内皮功能的影响及 PD 与 ADMA 之间的相互作用。**方法** 通过绘制主动脉条在 ADMA 与 PD 分别作用和共同作用时对苯肾上腺素 (PE) 的收缩量-效曲线, 计算并比较 E_{max} 和 K_d 值。**结果** 正常主动脉条对 ADMA 无反应, PD 或 ADMA 预处理后亦不影响其对 PE 的收缩反应。但 PD 使受 ADMA 预处理的主动脉条对 PE 的收缩反应呈剂量依赖性减弱, 并引起 K_d 值增大、E_{max} 减小。**结论** ADMA 或 PD 单独作用并不影响正常主动脉条的收缩功能及 PE 对 α-受体的亲和力和 E_{max}; 但在 ADMA 存在时, PD 能非竞争性拮抗主动脉条对 PE 的收缩反应。

关键词: 虎杖苷; 非对称性二甲基精氨酸; 一氧化氮合酶; 苯肾上腺素

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Endogenous NOS inhibitor—asymmetric dimethylarginine (ADMA) is a kind of methylated arginines. Recent studies show that its plasma level is significantly elevated in conditions including atherosclerosis and its related risk factors, such as hypertension, diabetes and hypercholesterolemia. There have been some reports on the change of vascular endothelial function caused by ADMA[1] and interaction of ADMA with vasoactive substances[2] in vascular contractive and dilative functions. Polydatin (3, 4′, 5-trihydrostilbene-3-β-*D*-glycoside, PD) was reported not only to have the effect of promoting calcium ion to enter vascular smooth muscle cell[3], but also to decrease the pressure of pulmonary artery[4]. There has been no report on the interaction between PD and ADMA on vascular contractive and dilative functions. This is the main aim of the present study.

1 Materials and methods

1.1 Drugs: Phenylephrine (PE, Hefeng Pharmacy, Shanghai), ADMA (Sigma), PD (Supplied by Prof. XIA Zhi-ling, from Chongqing University). *Polygonum cuspidatum* Sieb. et Zucc. was purchased from Chongqing Tongjunge Pharmacy. Its main components were polydatin (PD), emodin, anthraglycoside A, and chrysophanol. The PD content [(59.11±6.65)%, n = 15] was kept as the quality index of *P. cuspidatum*. PD is a kind of light yellow powder. It is easy to be damp and is dissolved in ion-free water before the experiment.

1.2 Tissue preparation: Five healthy male Japanese rabbits weighing 3.00—3.25 kg (purchased from Experimental Animal Centre of Chongqing University of Medical Sciences) were killed by air embolism. The thoracic aorta was cut out immediately and the connective tissue around it was removed. Then the aorta was placed into Krebs' solution which was kept at 37 ℃ and cut into spiral strip. The strips were preloaded with 2 g

weight and equilibrated for at least 1 h. During the whole experiment, the incubation system was aerated with a gas mixture of 95% O_2 and 5% CO_2. The change of aortic strip tension was recorded by LMS—2B physiology recorder.

1.3 Responses of aortic strips to different drugs

1.3.1 Cumulative dose-response curve of PE: After the equilibration period, different concentrations of PE were added into the bathtub. The final concentrations of PE were from 1×10^{-9} to 1×10^{-4} mol/L. The change of the tension in the strip was recorded simultaneously. E_{max} and EC_{50} were calculated according to Scatchard method.

1.3.2 Influence of ADMA on E_{max} and K_d induced by PE: In order to observe whether ADMA could induce the contraction of aortic strip, final concentrations of 1×10^{-6}, 1×10^{-5}, and 1×10^{-4} mol/L ADMA were added into the bathtub separately. Then the dose-response curve of PE was prepared, E_{max} and K_d were calculated according to the former method.

1.3.3 Influence of PD on E_{max} and K_d induced by PE: Different concentrations of PD (0, 1×10^{-6}, 1×10^{-5}, and 1×10^{-4} mol/L) were added into the bathtub, the influence of PD on the dose-response curve of PE was observed. E_{max} and K_d were calculated, too.

1.3.4 Effect of PD-pretreatment on function of aortic vascular strip in the presence of ADMA: Aortic strip was treated with various concentrations of PD (1×10^{-6}, 1×10^{-5}, and 1×10^{-4} mol/L), 1×10^{-5} mol/L ADMA, then the dose-response curve of PE was prepared, E_{max} and K_d were calculated.

1.4 Statistical analysis: Data were expressed as $\bar{x}\pm s$. Statistical analysis was done with SAS software package.

2 Results

2.1 E_{max} and K_d values of PE: E_{max} and K_d values were shown in Table 1.

2.2 Influence of ADMA on E_{max} and K_d induced by PE: Different concentrations of ADMA (1×10^{-6}, 1×10^{-5}, and 1×10^{-4} mol/L) couldn't induce normal aortic strip's contraction. Influence of ADMA on E_{max} and K_d induced by PE was shown in Table 1. It showed that ADMA didn't affect the aortic strip's contrative response to PE.

2.3 Influence of PD on E_{max} and K_d induced by PE: Neither could PD evoke any responses of healthy aortic strips, nor affect E_{max} and K_d values induced by PE. E_{max} and K_d values were shown in Table 2.

2.4 Effect of PD-pretreatment on function of aortic vascular strip in presence of ADMA: PD could significantly weaken the contraction of aortic strips pretreated with AMDA induced by PE in a dose-dependant manner. PD, 1×10^{-4} mol/L could increase K_d value significantly. It was suggested that PD can noncompetitively antagonize the α-receptor's contractive reaction to PE when PD and ADMA coexisted at the same time. E_{max} and K_d values were shown in Table 1.

Table 1 E_{max} **and** K_d **values of contractive response of aortic strips treated with ADMA and/or PD induced by PE** ($\bar{x}\pm s$, n= 5)

Groups	E_{max}	K_d/(1×10^{-7} mol·L^{-1})
PE(A)	47.67±20.05	7.04± 6.15
10^{-6} mol/L ADMA+PE(B)	58.80±19.03	5.62± 5.87
10^{-5} mol/L ADMA+PE(C)	49.98±16.12	11.80±10.99
10^{-4} mol/L ADMA+PE(D)	44.60±14.83※	18.31±17.21
10^{-6} mol/L PD+10^{-5} mol/L ADMA+PE(E)	16.89± 8.79*※★☆	21.01±11.04
10^{-5} mol/L PD+10^{-5} mol/L ADMA+PE(F)	10.08± 5.97*※★☆	29.53±41.41
10^{-4} mol/L PD+10^{-5} mol/L ADMA+PE(G)	3.07± 3.59*※★☆	33.36±26.24*※

*$P<0.01$ *vs* group A; ※$P<0.01$ *vs* group B; ★$P<0.01$ *vs* group C; ☆$P<0.01$ *vs* group D

Table 2 E_{max} **and** K_d **values of contractive response of aortic strips treated with PD induced by PE** ($\bar{x}\pm s$, n= 5)

Groups	PD/(mol·L^{-1})	E_{max}	K_d/(1×10^{-7} mol·L^{-1})
PE		61.29± 5.55	6.24±4.68
PD+PE	1×10^{-6}	78.12±10.09	5.67±3.45
	1×10^{-5}	73.36±11.69	7.82±5.67
	1×10^{-4}	62.34±10.86	7.38±5.01

3 Discussion

The present experiment was designed to confirm whether ADMA had effect on contractive function of aortic strips. The results showed that normal aortic strips did not respond to ADMA. When the strips were treated with ADMA, both E_{max} and K_d values induced by PE didn't change obviously. PE is an α-receptor agonist. The above

study showed that neither did ADMA cause resting tension of aortic strip, nor influence PE contractive efficiency and affinity to α-receptor on normal aortic strip. ADMA is an endogenous NOS inhibitor; some reports suggested that it can contract blood vessels, increase blood pressure[2], and decrease the release of NO from endothelial cells[1]. But the study did not support this. It has been reported that NO is a stress modulator for the tension of smooth muscle. But it is unclear whether NO could modulate blood vessel basal tension. The present study indicated that NO did not profoundly modulate blood vessel's basal tension.

PD is the main ingredient in *P. cuspidatum*. There have been many research reports about PD. But there are some paradox among them in its effect on smooth muscle and its modulation effect on calcium ion. JIN *et al.* reported that PD (in a concentration of 4×10^{-4} mol/L) could make normal smooth muscle cell membrane to be depolarized. This was related to both the opening of ion channel and the modulation of α-receptor, histamine receptor, and calcium channel[3]; PD not only promoted calcium ion to enter vascular smooth muscle cell, but also induced the release of intracellular calcium. PD might increase the contractility of normal vascular smooth muscle and the tension of normal vessel[5]. PD could also adjust vessel's tension by promoting extracellular sodium ion to flow inside[6]; PD can increase intracellular calcium concentration through the influx of extracellular calcium[7,8]. LI *et al.* reported that PD could decrease the pressure of pulmonary artery[4]. The results showed that PD could not evoke any response of rabbit aortic vessels when PD was used to vessels alone. When vessels were pre-treated with PD, PD could not change both E_{max} and K_d values of PE contracted by aortic strips significantly. These results suggested that PD did not exert a significant influence on the normal modulation and α-receptor-mediated modulation of smooth muscle's tension.

The study showed that PD could antagonize PE's contractive effects when it was used with ADMA simultaneously. It was manifest because E_{max} was decreased significantly and K_d values were increased significantly in a concentration-dependant manner. The present results showed that the inhibition of NOS caused by ADMA and the following decrease of NO were not enough to induce vessel contraction. PD could not only adjust the disordered NOS system in atherosclerosis, but also increase eNOS's activity (our unpublished data). When the vessel was exposed to ADMA and PD simultaneously, PD may restore the production of NO by endothelium, therefore strengthen vessel's dilative function. As a result, it antagonized the effect of PE on α-receptor. It could be deduced that PD could adjust the disordered NOS system. The importance was that PD did not significantly affect vessel's contractive and dilative function under normal circumstances; however, in conditions, such as atherosclerosis, then the concentration of plasma ADMA increased significantly, PD could exert its protecting effect on blood vessels from ADMA, antagonize PE's activating effect to α-receptor noncompetitively, dilate vessels, and improve organ's blood supply.

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