

## L-精氨酸能改善血管内皮依赖性舒张和抗动脉粥样硬化的损伤

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### L-Arginine Improves Endothelium-Dependent Vasorelaxation and Reduces the Development of Proliferative atherosclerotic lesions.

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**ABSTRACT** Reductions in nitric oxide(NO) activity persist after arterial intimal injury and may be a factor in the development of atherosclerosis. NO inhibits in vitro or in vivo platelet aggregation, leukocyte adhesion, and smooth muscle cell growth, all of which are key components in the process of intimal hyperplasia. We hypothesized that supplementation with L-arginine, the precursor of NO, would increase NO production and thereby improve endothelium-dependent vasorelaxation and inhibit the development of atherosclerosis. Twenty-four New Zealand white male rabbits were divided to four groups: ① uninjury, ② unilateral iliac artery injury + 2% cholesterol, ③ L-Arginine, ④ unilateral iliac artery injury + 2% cholesterol + 2% L-arginine. The iliac arteries were harvested for functional and morphometric studies. Maximal endothelium-dependent vasorelaxation in group ①, ③

and ④ was significantly greater than in group ②, but there was no significant difference of endothelium-dependent vasorelaxation among group ①, ③ and ④. L-arginine could partly inhibit the development of atherosclerotic plaques. These data suggest that L-arginine supplementation enhances NO production at sites of vascular healing and may reduce the development of proliferative atherosclerotic lesions.

**KEY WORDS** L-Arginine; Endothelium-dependent relaxation; Nitric Oxide; Atherosclerosis; Rabbits

**摘要** 内皮细胞功能不良、血小板和粒细胞的粘附和聚集、机械损伤的炎症反应、中膜平滑肌细胞向内皮下迁移增殖等在动脉粥样硬化发生中起重要作用,而内皮细胞持续产生释放的一氧化氮除介导血管内皮依赖性舒张作用外,补充L-精氨酸(一氧化氮的前体),将增加一氧化氮的产生和改进内皮依赖性舒张功能。本实验将24只新西兰兔分成4组:①未损伤组;②右侧髂动脉损伤+2%胆固醇组;③2%精氨酸组;④右侧髂动脉损伤+2%胆固醇+2%精氨酸组。结果发现,①、③和④组的内皮依赖性舒张功能值比②组大( $P < 0.01$ ),但前三组之间没有明显差别( $P > 0.05$ )。同时,L-精氨酸还能部分地抑制内皮剥脱和高胆固醇所引起家兔髂动脉内膜增厚。结果提示:补充L-精氨酸可能通过一氧化氮的产生增加,从而改善血管内皮依赖性舒张功能和抗动脉粥样硬化损伤的作用。

**关键词** L-精氨酸; 内皮依赖性舒张; 一氧化氮; 动脉粥样硬化

近年来大量的临床研究已证明,降低血浆胆固醇水平能使已形成的冠状动脉粥样硬化斑块消退,但其消退率仅为1%~2%,而临床上冠心病事件(不稳定型心绞痛、急性心肌梗塞和

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冠脉猝死)的发生率却减少30%~50%。显然,降胆固醇治疗所获的临床效益(即减少冠心病事件发生率),难以用冠脉斑块消退来解释。目前推测降胆固醇治疗的效益可能与血管内皮功能改善,使冠脉处于舒张状态,避免冠脉痉挛有关。目前认为,内源性一氧化氮(nitric oxide, NO)活性降低与冠心病的发生、发展有密切关系,补充NO前体即L-精氨酸对高胆固醇和内皮损伤家兔髂动脉内皮依赖性舒张功能及内膜增生有明显的影响<sup>[1]</sup>,本实验进一步探讨了L-精氨酸对实验性动脉粥样硬化家兔髂动脉内皮舒张功能和内膜增生的影响,为动脉粥样硬化的治疗提供理论依据。

## 1 材料和方法

### 1.1 动物模型

新西兰雄性白兔24只,体重2.0~2.4 kg,实验分四组:①未损伤组;②右侧髂动脉损伤+2%胆固醇组;③2%精氨酸组;④右侧髂动脉损伤+2%胆固醇+2%L-精氨酸组。动物进食100 g/天。损伤组行右侧髂动脉内皮剥脱术,以5%戊巴比妥钠0.4 ml/kg耳缘静脉注入麻醉,2Fr Fogarty导管自双侧股动脉逆行插入达膈水平,球囊内充以0.5~0.7 ml生理盐水,缓慢回拉导管至股动脉切口处,重复3次移出导管,结扎股动脉,继续喂养30天。

### 1.2 精氨酸水平测定

在实验前和动物处死时各收集一次耳静脉血液,分离血浆并贮存在-20℃,参照文献<sup>[2]</sup>经反相高效液相色谱测定精氨酸水平。

### 1.3 血清胆固醇测定

实验前1周,实验后第1、2、3和4周时分别测血清胆固醇。

### 1.4 血管内皮依赖性舒张

动物喂养高胆固醇和内皮剥脱后30天时,每组取2只动物静脉注射肝素500 U,戊巴比妥钠过量麻醉,迅速分离动脉并浸入氧合KH液( $\text{mmol} \cdot \text{L}^{-1}$ ): NaCl 118.1, KCl 4.7,  $\text{MgSO}_4$  0.56,  $\text{KH}_2\text{SO}_4$  12,  $\text{CaCl}_2$  5.0,  $\text{NaHCO}_3$  25.0和葡萄糖11.1, pH 7.4。剥离周围结缔组织,切成环状(4 mm长)。取2个动脉环,将其固定于无诱钢钩,于血管浴槽中,在新福林收缩的基础上,观察ACh诱导的舒张百分率。

### 1.5 光镜和电镜观察

在高胆固醇喂养和内皮损伤30天时,动物注入过

量戊巴比妥钠麻醉。每组选择5支髂动脉迅速取出, KH液冲洗,用10%福尔马林液固定,备作光镜标本。另每组随机取1只动物,先用KH液,再用2%戊二醛液作原位生理压灌流固定后,迅速取出髂动脉,备作电镜标本。光镜标本经固定30分钟后,HE染色,用IBS图象分析仪测定中膜面积(内弹性膜至外弹性膜之间的面积)和内膜面积(内弹性膜至血管腔面之间的面积)。扫描电镜按常规方法染色和观察。

### 1.6 资料分析

所有资料以平均数±标准误表示,统计分析用ANOVA方法。

## 2 结果

### 2.1 血浆精氨酸水平

实验前1周血浆各组精氨酸水平没有明显差别( $P>0.05$ )。实验后30天,喂养L-精氨酸组血浆精氨酸水平明显高于未用精氨酸组( $P<0.01$ , Figure 1)。

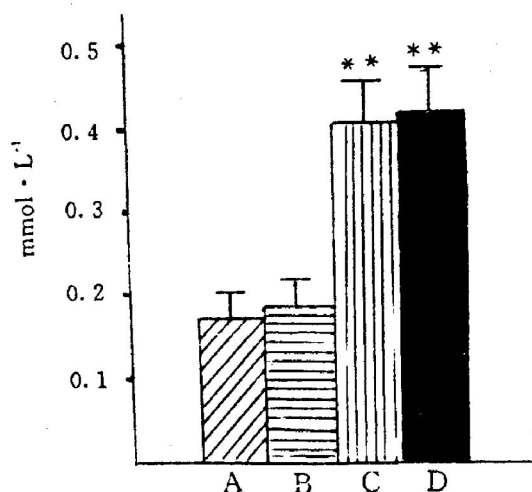


Figure 1. Plasma arginine levels were elevated in L-arginine-supplemented animals compared with control. A = no injury + no feeding cholesterol, B = injury + 2% cholesterol, C = L-arginine, D = injury + 2% cholesterol + 2% L-arginine. \*\*  $P<0.01$  compared with group A and B.

### 2.2 血清胆固醇水平

喂养胆固醇组动物在实验的第1、2、3和4周与未喂养胆固醇组相比,前者血清胆固醇明显增高,服L-精氨酸组对血浆胆固醇水平没有明显影响(Figure 2)。

### 2.3 内皮依赖性血管舒张功能

右侧髂动脉内皮剥脱+高胆固醇组与其它三组相比,前者血管内皮依赖性舒张值明显降

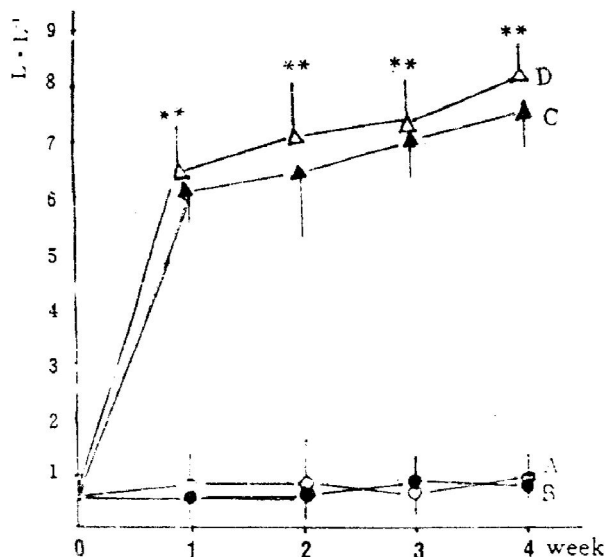


Figure 2. Plasma cholesterol levels of rabbit after arterial injury and 2% cholesterol. Plasma cholesterol level of rabbits after 2% cholesterol-feeding was significantly higher than that of rabbits feeding normal diets ( $P < 0.01$ ). A = no injury + no cholesterol + L-arginine, B = no injury + no cholesterol, C = injury + 2% cholesterol + 2% L-arginine, D = injury + 2% cholesterol.

低 ( $P < 0.01$ ), 右侧髂动脉内皮剥脱 + 高胆固醇 + L-精氨酸组、L-精氨酸组和未损伤组之间没有明显差别 ( $P > 0.05$ )。说明喂 L-精氨酸动物中内皮依赖性血管舒张功能较未用 L-精氨酸的好 (Figure 3)。

#### 2.4 光镜和电镜观察

光镜下见内皮剥脱 + 高胆固醇组家兔髂动脉内膜增生明显 (Figure 4), 内膜/中膜面积比值为  $1.37 \pm 0.15$ , 但内皮剥脱 + 高胆固醇 + L-精氨酸组内膜增生程度较内皮剥脱 + 高胆固醇组明显低得多 (Figure 5), 内膜/中膜面积比值为  $0.83 \pm 0.17$  ( $P < 0.01$ )。扫描电镜下观察内皮剥脱加胆固醇组的斑块表面的内皮细胞排列不规则, 斑块表面上的多数部位无内皮覆盖 (Figure 6); 内皮剥脱加胆固醇 + L-精氨酸组可见斑块覆盖着内皮细胞, 内皮细胞排列较规则 (Figure 7)。

#### 3 讨论

体内“L-精氨酸—一氧化氮”通路的发现源于对内皮源舒张因子 (endothelium derived relaxing factor, EDRF) 的研究, 目前已经证实体内广泛存在这一通路。近年来, 在阐明 NO 的合成机制、参与的酶系及其在不同系统内 NO 的

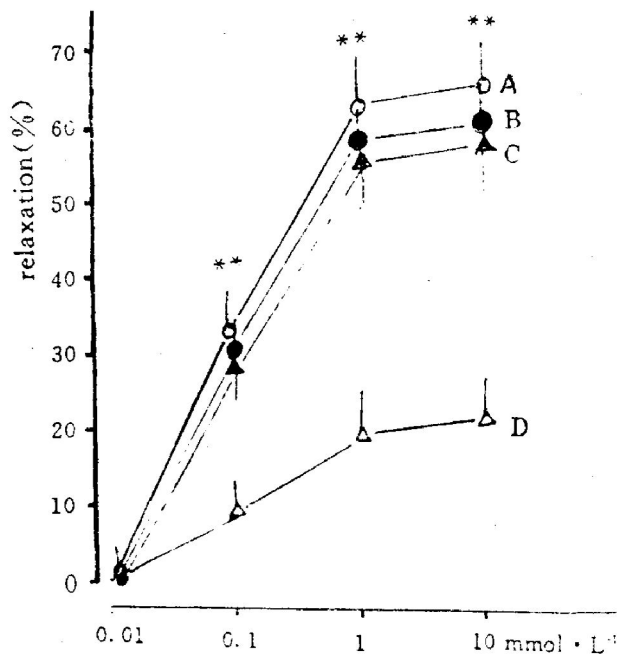


Figure 3. Endothelium-dependent relaxation in rabbit iliac arteries 30 days after arterial injury and 2% cholesterol. Responses are expressed as percent relaxation from precontracted baseline values. Endothelium-dependent vasorelaxation in the iliac arteries of rabbits with injury and feeding 2% cholesterol was significantly less than in iliac arteries of rabbits with feeding L-arginine. A = no injury + no cholesterol + L-arginine, B = no injury + no cholesterol, C = injury + 2% cholesterol + 2% L-arginine, D = injury + 2% cholesterol.

功能等方面有了很大的进展。且已明确, L-精氨酸为该通路的底物, 在一氧化氮合成酶 (nitric oxide synthase, NOS) 及其辅助因子的共同作用下, 其 N 端氨基脱氨氧化, 生成 NO 和 L-瓜氨酸 (L-citrulline)<sup>[3,4]</sup>。内皮细胞产生和释放 NO 受损会导致心血管功能异常, 与高脂血症、糖尿病和高血压发生发展密切相关<sup>[5~7]</sup>。Tarry 等<sup>[8]</sup>报道血管成形术后可使内皮细胞 L-精氨酸—NO 通路受损, 内皮依赖性舒张功能低下。血管成形术后内皮再生发生 2~3 周, 再生的内皮就出现形态学和功能的变化, NO 的合成和释放减少, 由此导致内皮依赖性舒张障碍。我们实验显示, 服用 L-精氨酸 (NO 的前体) 可使高脂饮食和内皮剥脱的家兔髂动脉内皮依赖性舒张恢复至正常状态, 内膜增生程度减轻, 泡沫细胞的形成减少; 电镜下可见喂养

L-精氨酸组斑块表面的内皮排列比未喂饲 L-精氨酸组的整齐。这些结果说明 L-精氨酸具有明显抗动脉粥样硬化的作用。

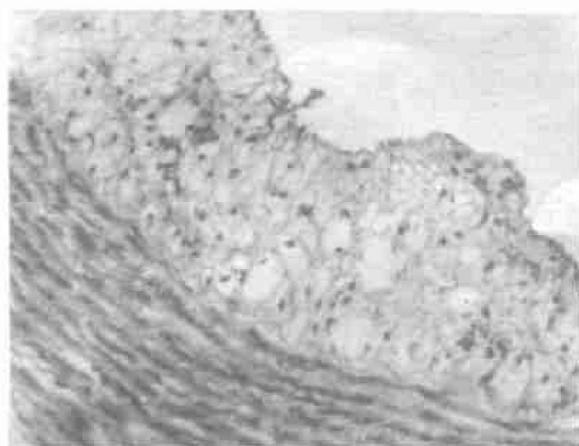


Figure 4. Photomicrograph of iliac artery in injury + 2% cholesterol group. Hematoxylin-eosin stain, original magnification  $\times 40$ .



Figure 5. Photomicrograph of iliac artery in injury + 2% cholesterol + 2% L-arginine group. Hematoxylin-eosin stain, original magnification,  $\times 40$ .



Figure 6. Scanning electron microscopy of injury + 2% cholesterol group.  $\times 200$ .



Figure 7. Scanning electron microscopy of injury + 2% cholesterol + 2% L-arginine group.  $\times 200$ .

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