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科素亚对长期大强度运动所致大鼠心脏纤维化的预防作用*

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摘要 目的:研究血管紧张素 II 1 型受体拮抗剂科素亚对长期大强度运动所致心脏纤维化的预防作用。**方法:**48 只雄性 Wistar 大鼠随机分为 4 组:对照组,科素亚处理的对照组,运动组,运动+科素亚处理组。运动组大鼠给予 16 周大强度运动,科素亚处理的大鼠每天训练前口服科素亚每次(50 毫克/公斤/天)。通过计算心系数和观察心脏的组织形态学评估和比较各组大鼠心脏肥大的程度,免疫印迹法检测和比较各组大鼠 4 个心腔内转化生长因子- β 1、纤维连接蛋白 1、基质金属蛋白酶-2、I 型胶原蛋白和 III 型胶原蛋白的蛋白表达水平。**结果:**长期大强度运动可造成大鼠左心室心脏壁肥大和右心室胶原沉积,心房和右心室的主要纤维化生物标志物的蛋白表达水平显著增加。科素亚预处理能够减少主要纤维化生物标志物 TGF- β 1、FIBROECTIN-1、MMP-2、TIMP-1、COLLAGEN-I、COLLAGEN-III 表达水平,但不能完全改变心脏重量与体重比值增大的状态。**结论:**科素亚可部分预防耐力运动训练所致的心脏纤维化,但不能彻底改善心脏肥大。

关键词:科素亚;长期;大强度运动;心脏纤维化

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Preventive Effect of Cozaar on the Long-Term Intensive Exercise Induced Heart Fibrosis in rats *

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ABSTRACT Objective: This study examined the antifibrotic effect of Cozaar, an angiotensin II type 1 receptor antagonist, in an animal model of heart fibrosis induced by long-term intense exercise. **Methods:** Male Wistar rats were randomly distributed into 4 experimental groups: Exercise, Exercise plus losartan, Sedentary and Sedentary plus Cozaar. Exercise groups were conditioned to run vigorously for 16 weeks. Cozaar was orally administered daily before each training session (50 mg/kg/day). After euthanasia, heart hypertrophy was evaluated by histological studies; ventricular collagen deposition was quantified by histological and biochemical studies; and protein expression of transforming growth factor- β 1, fibronectin-1, matrix metalloproteinase-2, tissue inhibitor of metalloproteinase-1, procollagen-I and procollagen-III was evaluated in all 4 cardiac chambers. **Results:** Daily intensive exercise caused hypertrophy in the left ventricular heart wall and originated collagen deposition in the right ventricle. Additionally long-term intensive exercise induced a significant increase in protein synthesis of the major fibrotic markers in both atria and in the right ventricle. Cozaar treatment was able to reduce all increases of protein synthesis of the major fibrotic markers, although it could not completely ameliorate the the ratio between body and heart weight caused by intensive exercise. **Conclusion:** Cozaar treatment prevents the heart fibrosis induced by endurance exercise in training animals, however, it could not completely reverse the heart hypertrophy.

Key words: Cozaar; Longterm; Intensive exercise; Heart Fibrosis

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研究表明,长期大强度的运动可能增加心律失常发生的风险^[1-5],可导致促纤维化心肌的重塑^[6,7],但这些改变的发生机制及临床意义目前尚未完全阐明。长期运动可导致心脏的血流动力学改变及心脏的负荷条件。心脏血流动力学过载可激活肾素-血管紧张素系统(renin angiotensin aldosterone system, RAS)^[8,9],进一步通过影响血管张力、体液和电解质平衡等机制维持心血管稳态,并可诱导成纤维细胞的增殖和肥大^[10,11]。血管紧张素 II (ANGII)由其前体血管紧张素 I 经血管紧张素转换酶(ACE)的

蛋白水解产生,通过激活血管紧张素型 I 受体(AT1)^[13,14]在心肌纤维形成中起着重要的作用^[12]。科素亚是一种选择性 AT1 受体拮抗剂,可通过抑制心脏 RAS 系统,改善左心室功能,防止几何重构^[15,16],还可发挥抗肺纤维化的作用^[17]。本研究旨在利用长期耐力运动诱导心脏纤维化的动物模型评估科素亚预防心脏纤维化的作用。

1 材料与方法

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1.1 实验动物及分组

4 周龄雄性清洁级 Wistar 大鼠 48 只,体重 100-125 克,由本校实验动物中心提供。将动物随机分成 4 个实验组:对照组(C 组),科素亚处理的对照组(L 组),运动组(E 组)以及科素亚处理的运动组(E+L 组),每组 12 只大鼠。运动负荷:开始跑台斜度为 6%,速度 15 米/分,每天 15 分钟,5 天后逐渐增加负荷,分别隔日增加速度 2 米/分,时间增加 5 分钟,最终速度达 35 米/分,运动时间 60 分钟时,再将跑台斜度增至 10%,整个训练期每周训练 5 天,连续 16 周。科素亚(50mg/kg/d)灌胃给药,药物溶解在最终体积为 1.5 毫升的蒸馏水中,每天运动训练开始前给药。

1.2 心肌肥厚的评价

训练结束后 24h,大鼠断头处死,迅速取出心脏,称重,解剖左心室(LV),右心室(RV),左心房(LA),右心房(RA),液氮中冷冻储存,计算心脏重量与体重比值评估大鼠心肌肥厚的程度。

1.3 组织学观察

心脏组织块依次浸入 10%、20%、30%的蔗糖溶液脱水。然后经 OCT 包埋,行连续恒冷箱切片,厚度 16 μm ,组织切片经水化后,Picosirius red 染色,梯度酒精脱水,二甲苯透明,中性树胶封片,光学显微镜(OLYMPUS, BX51)下观察。

1.4 免疫印迹法检测纤维环相关蛋白的表达

各部位心脏组织加入蛋白匀浆液(50mM This-HCl(pH7.4), 1% Triton X-100, 0.2 mM PMSF, 1 mM EDTA),用 4℃ 超速离心机 12000 r/min,离心 10 min,取上清,用 10%的聚丙烯酰胺凝胶电泳分离蛋白,10 $\mu\text{g}/\text{well}$,电泳结束后,将蛋白从聚丙烯酰

胺凝胶转移到 PVDF 膜上,TGF- β 1(1:1000)或 FIBROECTIN-1(1:1000)或 MMP-2(1:1000)TIMP-1(1:1000)或 COLLAGEN-I(1:1000)或 COLLAGEN-III(1:1000)或 -actin(1:2500)一抗孵育,4℃ 过夜,加标记辣根过氧化物酶的抗鼠/兔 IgGII 抗,常温孵育 1h,然后用 ECL 发光,X 光胶片曝光成像,TotalLab 软件进行图像分析。一抗来源:TGF- β 1(Santa Cruz, Santa Cruz, CA), FIBROECTIN-1(Santa Cruz, Santa Cruz, CA), MMP-2(Santa Cruz, Santa Cruz, CA), TIMP-1(Santa Cruz, Santa Cruz, CA), COLLAGEN-I(Santa Cruz, Santa Cruz, CA), COLLAGEN-III(Santa Cruz, Santa Cruz, CA), β -actin(北京中山金桥公司)。

1.5 统计学分析

实验结果以均数 $\pm$ 标准差($\bar{x} \pm s$)表示,采用 SPSS13.0 统计软件(SPSS Inc, USA),组间比较应用方差分析,两组之间的比较采用 SNK-q 检验,以 $P < 0.05$ 为差异具有统计学意义。

窗体顶端

2 结果

2.1 各组大鼠心肌肥厚程度的比较

E 组大鼠体重较 C 组明显减轻($P < 0.05$);而 E+L 组大鼠体重与 L 组比较显著降低($P < 0.05$),而与 E 组比较无显著性差异;L 组体重与 C 组相比虽有下降趋势,但二者差异无统计学意义。所有组别之间心脏重量比较未见明显差异。而 E 组心脏重量与体重比与 C 组相比显著升高 ($P < 0.05$);E+L 组心脏重量与体重比与 L 组相比也显著升高($P < 0.05$),而与 E 组比较无显著性差异;L 组体重与 C 组相比,二者差异无统计学意义。见表 1。

表 1 运动及科素亚对体重、心脏重量和二者比值的影响($\bar{x} \pm s$, n=6)

Table 1 The effects of exercise and cozaar on body weights, heart weights and heart weight/body weight ratios

组别	体重(g)	心脏重量(g)	心脏重量/体重(%)
C	414.8 $\pm$ 13.98	1.03 $\pm$ 0.09	0.24 $\pm$ 0.01
L	382.5 $\pm$ 17.97	0.94 $\pm$ 0.09	0.25 $\pm$ 0.03
E	357.1 $\pm$ 8.25*	1.12 $\pm$ 0.05	0.31 $\pm$ 0.01*
E+L	339.7 $\pm$ 8.96#	1.03 $\pm$ 0.04	0.31 $\pm$ 0.00#

注:与 C 组相比,* $P < 0.05$;与 L 组相比,# $P < 0.05$ 。

Note: * $P < 0.05$ E group vs. C group; # $P < 0.05$ E+L group vs. L group.

2.2 各组大鼠心脏组织形态学的比较

如图 1,紫红色染色为间质型胶原,C 组、L 组以及 E+L 组均未见间质性胶原沉积,而 E 组可见弥漫性间质性胶原沉积以及心肌结构排列紊乱,胶原蛋白的密度显著增加,心肌结构正常。

2.3 各组大鼠心肌纤维化标志物蛋白表达的比较

进一步应用免疫印迹法分析了心脏不同部位纤维化主要生物标志物 TGF- β 1、Fibroectin-1、MMP-2、TIMP-1、Collagen-I、Collagen-III 的表达变化。E 组大鼠心房和右心室 TGF- β 1、FIBROECTIN-1、MMP-2、TIMP-1、COLLAGEN-I、COLLAGEN-III 的蛋白表达水平与 C 相比均显著增加 ($P < 0.05$),而 E+L 组 TGF- β 1、FIBROECTIN-1、MMP-2、TIMP-1、COLLAGEN-I、COLLAGEN-III 的蛋白表达水平较 E 组均显著减少($P < 0.05$),与对

照组无显著差异($P > 0.05$)(图 2A-F)。以上结果表明,大强度耐力运动 16 周后心肌细胞外基质(ECM)发生显著变化,在 RV 而不是 LV 发生明显的纤维化;而科素亚可预防大强度运动所致的心脏重塑。

3 讨论

运动性心脏肥大是心脏对运动训练的适应性反应,是一种生理性心脏重塑的过程。长期运动可引起血流动力学以及心脏负荷状态的改变^[9],进而使心脏产生生理适应性结构重塑^[18]。而长期大强度运动可诱发心脏重塑和纤维化,最终导致心脏组织的功能障碍,如心脏衰竭和高血压^[19,20]。心系数为心脏重量与体重的比值,是反映心肌肥大的重要指标。本实验结果显示,长期耐力训练后大鼠心脏重量及心系数均较正常大鼠显著增加,表

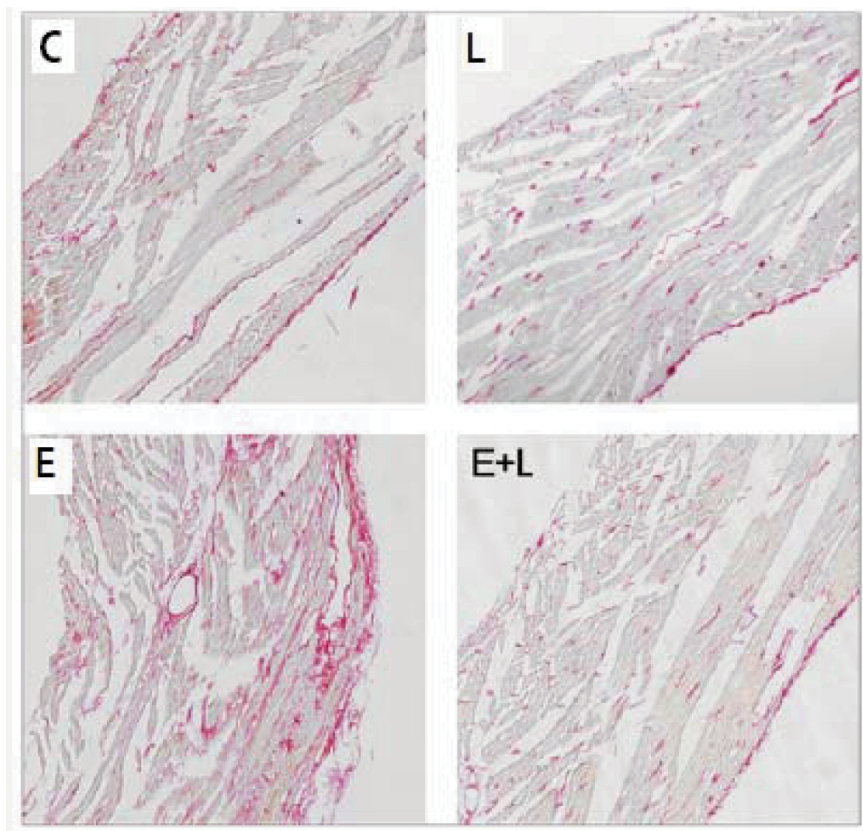


图1 运动及科素亚对心肌组织形态学的影响(× 200)

Fig.1 The effects of exercise and cozaar on myocardium histomorphology (× 200)

明长期耐力训练可诱导运动性心肌肥大的发生。

研究表明,急性大强度运动可引发反应性瘢痕组织形成以及细胞坏死和纤维化生物标志物水平的急剧升高^[2]。Benito 等的研究表明,16 周的耐力运动训练可诱发大鼠左心室肥厚,两个心房和右心室心肌纤维化以及细胞外基质蛋白合成的改变^[6]。本研究结果也表明长期大强度运动可导致仅限于 LV 的心肌肥厚发生,而 RA、LA 和 RV 等部位心肌组织中纤维化表达和合成的标记物水平却显著升高。长期运动训练所导致的心脏上述变化特点,可能使相对更薄壁更容易受到心脏容积和压力超负荷损害,而压力和体积的过载可进一步增加左室侧壁的厚度。长期大强度运动所致的细胞外基质组合物改变可能会增加心肌的僵硬程度,引发舒张功能障碍,进而会增加心律失常的发生风险^[9]。

心肌重塑的病理改变包括心肌肥厚、心肌细胞死亡以及间质的动态变化。间质性改变涉及纤维状的胶原蛋白网络的变化(主要是 I 型和 III 纤维状胶原蛋白)^[20]。I 型胶原决定心肌的刚度,而 III 型胶原具有较好的延展性。本研究结果显示,长期大强度运动可显著增加右心室 I、III 型胶原蛋白表达水平,其中 I 型胶原的表达上调更加突出,表明长期大强度运动可以增加心脏的刚度。

TGF- β 1 等细胞因子可诱导心脏成纤维细胞发生向成纤维细胞的表型变化,进而促进了胶原蛋白的沉积^[21]。血管紧张素 II 在刺激心脏进行性重塑和促进心肌纤维化中扮演极其重要的角色,可通过 TGF- β 1 的表达间接诱发心肌纤维化。心肌胶原蛋白的含量是受到胶原蛋白的产生和降解生理平衡过程的严格调控。基质金属蛋白酶(MMPs)可调控胶原蛋白代谢及细

胞外分解过程。本研究结果显示,长期大强度运动可显著增加心房和右心室 TGF- β 1、Fibroectin-1、MMP-2、TIMP-1、Collagen-I、Collagen-III 的蛋白表达水平,进一步表明长期大强度运动可增加胶原蛋白合成、沉积以及纤维化发展。

AT1 受体拮抗剂具有抗心肌纤维化和增生的作用^[6],可减轻左心室肥厚^[20],抑制 TGF- β 1 的早期诱导表达和心肌纤维化的后续发展^[21]。科素亚是一种选择性 AT1 受体拮抗剂,可阻断 RAS 系统。本研究结果表明,科素亚能够部分地防止长期大强度运动模型动物左心室肥厚的发生;同时,科素亚能够有效改善耐力运动所致的纤维化标志物水平的异常改变,从而防止心肌细胞外基质重塑。

综上所述,长期大强度运动可能与左心室肥厚和心肌间质纤维化的发生密切相关,而科素亚可通过抑制心脏 RAS 系统减轻耐力运动引发的心脏纤维化。本研究结果为科素亚抗纤维化作用提供了进一步的实验证据。

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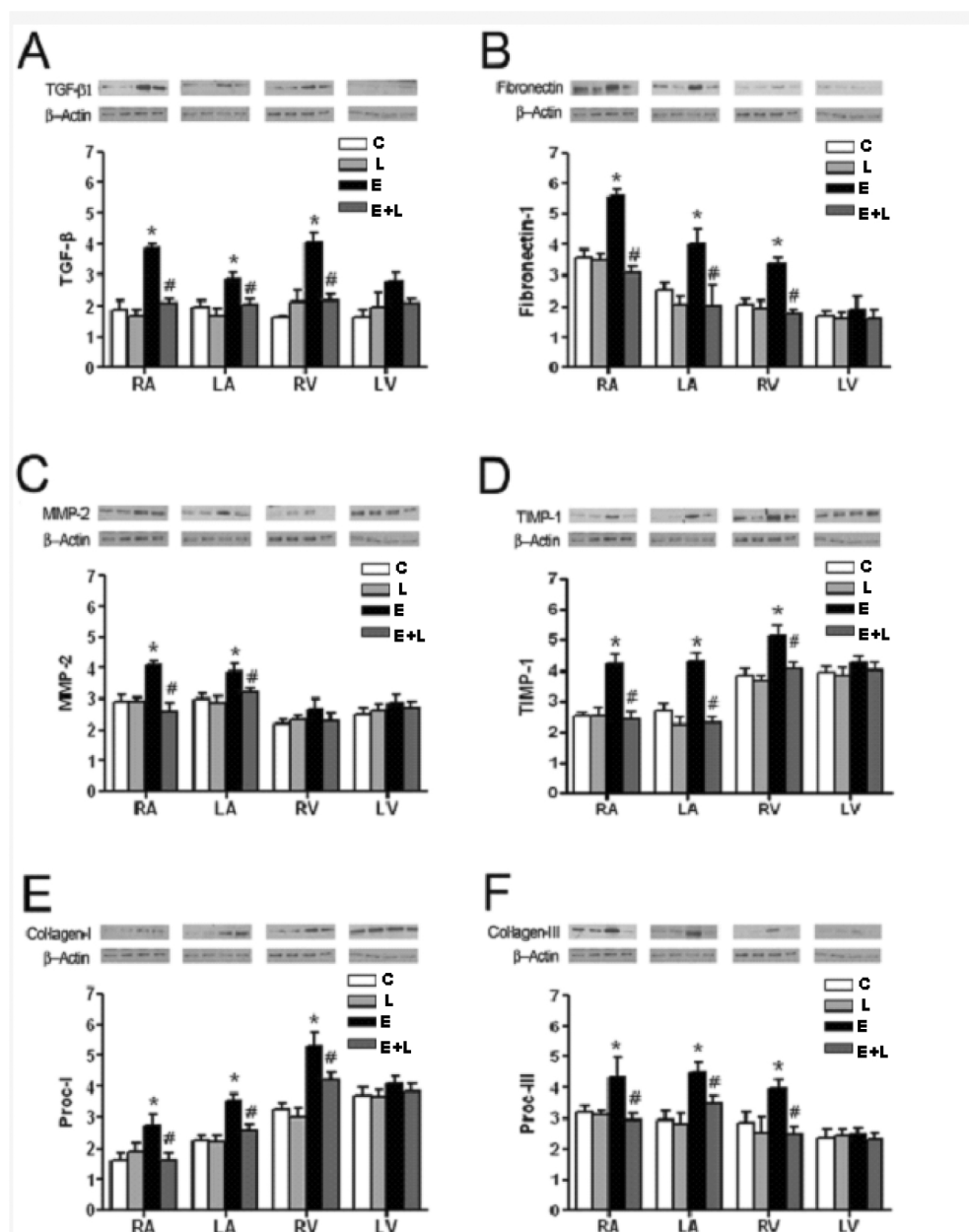


图2 运动及科素亚对心肌纤维化主要生物标志物表达的影响(与C组相比,* $P<0.05$;与E组相比,# $P<0.05$, $n=6$)

Fig.2 The effects of exercise and cozaar on expression of the major fibrotic markers(* $P<0.05$ E group vs. C group; # $P<0.05$ E+L group vs. E group; $n=6$)

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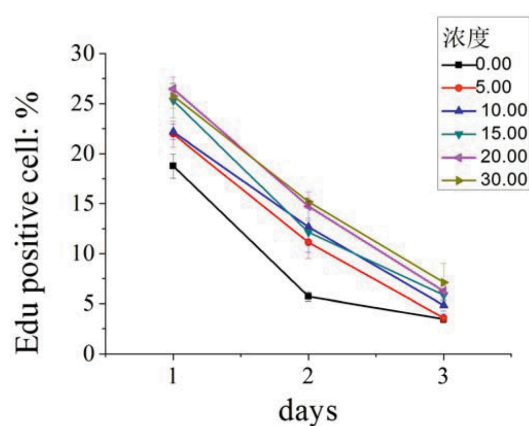


图 7 IGF-1 促进 ADSCs 增殖的量效关系

Fig.7 IGF-1 promotes proliferation of ADSCs with dose-effect relationship

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(上接第 2639 页)

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