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· 研究快报 ·

羟乙基淀粉130/0.4改善大鼠心肌缺血再灌注损伤

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[摘要] **目的**:观察羟乙基淀粉(HES)130/0.4(万汶)对缺血再灌注(I/R)损伤心肌的干预作用,探讨其可能的作用机制。**方法**:24只SD大鼠随机均分为4组(n=6):假手术(S)组、缺血再灌注(IR)组、白蛋白-缺血再灌注(A-IR)组、HES-缺血再灌注(H-IR)组。后3组建立在体大鼠心肌I/R模型,分别在缺血25 min时股静脉持续泵入生理盐水、5%白蛋白或HES 130/0.4,假手术组行开胸手术但不结扎左冠状动脉前降支。再灌注180 min处死大鼠,取心肌组织观察病理学改变;处死前颈动脉采血ELISA法测定TNF-α、IL-1β浓度;测定心肌组织NF-κB活性。**结果**:H-IR组心肌组织损伤病理学改变较IR组、A-IR组减轻。IR组、A-IR组、H-IR组血清TNF-α、IL-1β水平和心肌NF-κB活性明显高于S组(P<0.05),但其中H-IR组血清TNF-α、IL-1β水平及心肌NF-κB活性上升程度不及IR组、A-IR组(P<0.05)。**结论**:羟乙基淀粉130/0.4能减轻缺血再灌注所致心肌病理损伤,可能与其抑制NF-κB的活性、减少促炎细胞因子的释放有关。

[关键词] 心肌再灌注损伤;肿瘤坏死因子α;白细胞介素1β;NF-κB;羟乙基淀粉
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Protective effects of hydroxyethyl starch 130/0.4 against myocardial ischemia-reperfusion injury in rats

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[**ABSTRACT**] **Objective**: To explore the influence of hydroxyethyl starch(HES) 130/0.4 on myocardial ischemia-reperfusion (I/R) injury in rats and the possible mechanism. **Methods**: Twenty-four SD rats were evenly randomized into four groups(n=6): the sham operation group, the I/R(IR) group, albumin + I/R(A-IR) group, and HES+I/R(H-IR) group; rats in the latter three groups were made into I/R models and were treated respectively with 7.5 ml/kg saline, 5% albumin and HES 130/0.4 through femoral vein at 25 min of ischemia. At 180 min of reperfusion, animals were sacrificed and the pathological changes of myocardium were observed. Serum concentrations of TNF-α and IL-1β and the myocardial NF-κB activity were also measured. **Results**: Histological examination showed that the injury in H-IR group was ameliorated compared with those in IR and A-IR groups. NF-κB activity and TNF-α, IL-1β concentrations in the sham operation group were significantly lower than those of the other 3 groups (P<0.05); and the increases of the above parameters in H-IR group were smaller than those of the IR and A-IR groups (P<0.05). **Conclusion**: HES 130/0.4 can improve myocardial function and attenuate ischemia-reperfusion injury, and the mechanism might be related to the inhibition of myocardial NF-κB activity and reduction of proinflammatory factors.

[**KEY WORDS**] myocardial reperfusion injury; tumor necrosis factor-alpha; interleukin-1beta; nuclear factor κ-B; hetastarch
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缺血再灌注(I/R)损伤与炎症反应密切相关,I/R可导致一系列炎症因子(TNF-α、IL-1β等)的释放,激活中性粒细胞(PMN),加重对再灌注组织的浸润和损伤[1]。核转录因子NF-κB广泛存在于哺乳动物细胞中,参与机体的多种应激反应,在心肌I/R损伤中发挥着重要作用[2]。活化的NF-κB从胞质转移入胞核,与靶基因的κB位点结合,促进炎性介质表达增加[3],导致炎症反应、细胞坏死和细胞凋亡,加重组织器官功能的损伤。

羟乙基淀粉(HES)130/0.4(万汶)具有良好的

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扩容效应、持续时间及明显的抗炎作用[4]，广泛用于手术、创伤、失血、脓毒症等危重患者的容量替代治疗和液体复苏治疗。我们前期研究[5]亦证实其具有抑制 PMN 激活和浸润，促进 I/R 损伤心脏心功能恢复，保护心肌的作用。本研究进一步观察其对 I/R 损伤大鼠 NF-κB 和相关炎症因子表达及 I/R 损伤心肌病理学改变的影响，深入探讨其心肌保护作用及可能机制，为后续研究及临床应用提供理论依据。

1　材料和方法

1.1　*主要试剂及仪器*　HES 130/0.4 由 Fresenius Kabi Deutschland Gmbh 生产，北京费森尤斯卡比医药有限公司分装；TNF-α、IL-1β 检测试剂盒购自上海华涛生物技术有限公司；非放射性 NF-κB p65 转录因子活性试剂盒购自上海康成生物技术有限公司；H-500 透射电镜为日本 Hitachi 公司产品；ALC-V8 动物呼吸机为上海奥尔科特生物科技有限公司产品；WZS-50F 微量注射泵为浙江浙大医学仪器有限公司产品。

1.2　*动物分组及处理*　Sprague-Dwley 大鼠 24 只购自中国科学院上海实验动物中心，体质量(240±20) g，雌雄不拘，随机分为 4 组：假手术(S)组(n=6)不结扎左冠状动脉前降支(LAD)，仅持续输注生理盐水 7.5 ml/kg；后 3 组为缺血再灌注(IR)组、白蛋白-缺血再灌注(A-IR)组、HES-缺血再灌注(H-IR)组(n=6)，参照文献[6]制备 I/R 损伤模型，分别于 LAD 结扎 25 min 从股静脉以 0.2 ml/min 持续泵入生理盐水、5% 白蛋白或 HES 130/0.4，剂量均为 7.5 ml/kg，观察 180 min。各组大鼠再灌注结束后颈动脉采血，ELISA 法测定 TNF-α、IL-1β 水平；断头处死后，取 LAD 结扎处以下新鲜心肌组织置于－80℃冰箱保存，观察心肌病理学改变及测定 NF-κB 活性。

1.3　*各指标的观察*

1.3.1　*心肌组织超微结构*　每组随机取 3 只大鼠，取左心室同一部位的心肌内壁组织 0.1 cm×0.1 cm 大小 3 块，放入 4% 多聚甲醛液(电镜专用)冷藏固定，透射电镜观察心肌组织超微结构的变化。

1.3.2　*血清细胞因子测定*　再灌注结束后抽取颈动脉血 3 ml 于洁净试管中，离心分离血清，采用 Multiskan Ascent 酶标仪 ELISA 法测定 TNF-α、IL-1β 浓度，具体方法参照试剂盒说明书。

1.3.3　*心肌组织 NF-κB 活性检测*　提取心肌组织核蛋白，按照试剂盒说明进行操作，用酶标仪检测光密度 D_{450} 值代表 NF-κB 活性。

1.4　*统计学处理*　采用 SAS 9.1.3 软件包进行分析，计量资料以 $\bar{x}\pm s$ 表示，多组间均数比较用重复测量的方差分析，正态分布的计数资料组间用 SNK 法比较。

2　结　果

2.1　*心肌超微结构改变*　S 组：心肌肌原纤维排列整齐，线粒体完整，嵴排列整齐，糖原颗粒丰富，核染色体分布均匀(图 1A)；IR 组和 A-IR 组：心肌肌原纤维排列紊乱，肌丝有部分灶性溶解，肌质网和线粒体肿胀、基质疏松、嵴排列紊乱(图 1B、1C)；H-IR 组：心肌细胞膜、细胞核基本正常，无明显细胞水肿，肌原纤维较整齐，有个别松散区，线粒体均匀分布于肌原纤维之间，膜较完整，嵴致密，偶见空泡形成(图 1D)。

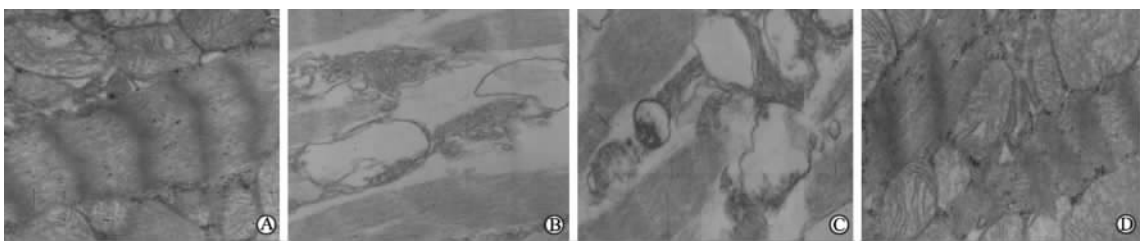

图 1　各组心肌组织超微结构的改变

Fig 1　Ultrastructure of myocardial tissues in different groups

A: In S group, muscle fibrils and cristae were arranged in order, cytochondriome was complete, there were abundant glycogen granules, and the nuclear chromatosome was well-distributed. B, C: In I/R and A-I/R group, cardiac muscle fibrils and cristae were in disorder, there were focus dissolves in myofilaments, sarcoplasmic reticulum and cytochondriome were swollen, and the basilaris substantia was loose. D: In HES+I/R group, the cardiac cell membrane and nuclear were fundamentally in good status, there was no obvious cellular edema, muscle fibrils were arranged in order with individual loose spots, cytochondriome was well-distributed in muscle fibrils, cell membrane was complete and cristae was arranged in order, and several vacuolations were seen. Original magnification: ×15 000

2.2　*大鼠血清细胞因子的变化*　结果(表 1)表明：再灌注 180 min，IR 组、H-IR 组和 A-IR 组大鼠血清 TNF-α、IL-1β 浓度显著高于 S 组(P<0.05)，但 H-IR 组血清 TNF-α、IL-1β 水平上升程度不及 IR 组、

A-IR 组($P<0.05$),IR 组、A-IR 组无统计学差异。

2.3 大鼠心肌组织 NF-κB 活性的变化 结果(表 1)表明:再灌注 180 min,IR 组、A-IR 组和 H-IR 组大鼠心肌组织 NF-κB 活性显著高于 S 组($P<0.05$),但 H-IR 组心肌组织 NF-κB 活性上升程度不及 IR 组、A-IR 组($P<0.05$),IR 组、A-IR 组无统计学差异。

表 1 大鼠血清细胞因子及心肌组织 NF-κB 活性的变化

Tab 1 Changes of serum cytokine concentration and myocardiac NF-κB activity

($n=6, \bar{x}\pm s$)

Group	TNF-α ρ_B/(ng·ml^{-1})	IL-1β ρ_B/(ng·ml^{-1})	NF-κB activity (D_{450} value)		
			Bioin labeled p65	Unlabeled wild p65	Unlabeled mutant p65
S	0.07±0.01	29.2±5.81	0.567±0.120	0.539±0.090	0.589±0.122
IR	1.81±0.18*	140.8±19.4*	1.989±0.392*	0.527±0.089▲	1.973±0.411
A-IR	1.78±0.19*	139.8±21.1*	1.970±0.408*	0.582±0.094▲	2.003±0.362
H-IR	1.02±0.17*△	93.9±15.3*△	1.486±0.249*△	0.548±0.103▲	1.452±0.253

*$P<0.05$ *vs* S group; △$P<0.05$ *vs* IR group; ▲$P<0.05$ *vs* bioin labeled p65 in the same group

3 讨 论

I/R 损伤本质上是一种强烈的炎性损伤过程,心脏 I/R 损伤模型 I/R 期间 NF-κB 的活化可能与 LAD 阻断及再灌注的无复流现象致心肌处于低氧状态有关[6-7]。炎症细胞因子 TNF-α、IL-Iβ 水平的增加和缺氧均可激活 NF-κB,活化的 NF-κB 迅速进入核内启动炎症细胞因子、选择素和黏附分子的表达,再升高的 TNF-α、IL-1β 等进一步刺激 NF-κB,形成正反馈,导致细胞因子水平不断升高[8]。抑制 NF-κB 的激活在 I/R 引起的心肌损伤中可能起一定程度的保护作用[2]。因此,本研究尝试应用 HES 130/0.4 抑制 NF-κB 活化,抑制炎症反应,拮抗心肌 I/R 损伤。

本研究结果发现 H-IR 组心肌 NF-κB p65 活性显著低于 IR 组和 A-IR 组,血清 TNF-α、IL-1β 浓度较 IR 组和 A-IR 组降低($P<0.05$),证实 HES 130/0.4 可抑制 NF-κB 活化,保护心肌功能。其发挥心肌保护作用的机制与如下几个方面有关:(1)HES 130/0.4 可通过抑制内皮活性,阻止中性粒细胞与黏附分子的黏附,从而降低中性粒细胞与内皮的黏附作用[9-11]。(2)HES 130/0.4 可减少促炎细胞因子的释放[2]。(3)HES 130/0.4 可抑制中性粒细胞的呼吸爆发[12]。(4)HES 130/0.4 可抑制 NF-κB 的活性[6]。

综上所述,羟乙基淀粉 130/0.4 能减轻缺血再灌注所致心肌病理损伤,可能与其抑制 NF-κB 的活性、减少促炎细胞因子的释放有关。

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