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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)。

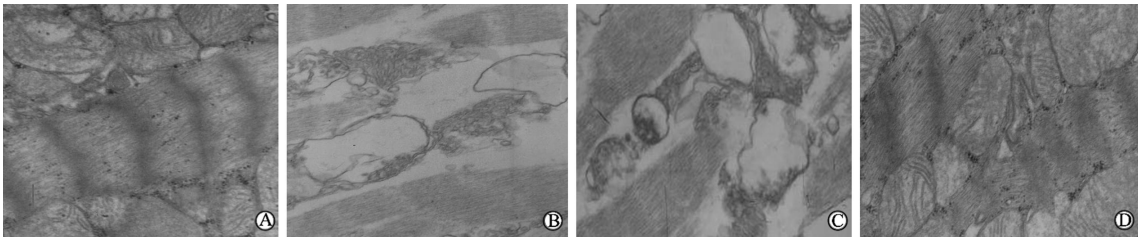

图 1　各组心肌组织超微结构的改变

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-1β 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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