·基础研究·

缺血后处理对大鼠缺血/再灌注心肌热休克蛋白的影响

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摘要 **目的:**研究缺血后处理对大鼠缺血/再灌注心肌热休克蛋白(HSP70)的影响。**方法:**选择健康 SD 大鼠 48 只,随机分为 3 组:假手术组、缺血再灌注组(对照组)和缺血后处理组,每组 16 只。制备大鼠心肌缺血/再灌注模型。缺血再灌注组,收紧结扎线缺血 40 min, 放松结扎线再灌注 240min;缺血后处理组,缺血 40 min 后, 再灌注 10s,缺血 10s,连续 3 个循环,然后再灌注 240min;假手术组,开胸后穿线做套环,但不收紧结扎线。免疫组织化学染色检测 HSP70 的表达,TUNEL 法检测心肌细胞凋亡指数,同时测定血清肌酸激酶活性。**结果:**①血清肌酸激酶活性测定:再灌注结束后缺血后处理组和缺血再灌注组肌酸激酶活性明显高于假手术组, 分别为 (712.13±42.77),(935.17±57.99),(282.74±29.54)U/L,P<0.05,缺血后处理组明显低于对照组(P<0.05)。②心肌凋亡细胞计数:再灌注结束后假手术组未见明显细胞凋亡 (<5%), 缺血后处理组心肌细胞凋亡率明显低于缺血再灌注组, 分别为 (14.3±2.7)%,(22.3±3.6)%,(P<0.05)。③心肌热休克蛋白表达:缺血后处理组较对照组心肌热休克蛋白表达增强(P<0.05)。**结论:**缺血后处理可减轻缺血再灌注损伤,其机制可能与增强热休克蛋白表达,减少心肌细胞凋亡有关。

关键词 再灌注损伤;热休克蛋白;凋亡

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Effects of postconditioning on the expression of heat shock protein 70 in rats with myocardial ischemia reperfusion/LIU Shenghui, YIN Boying, HUO Yu′e//Chinese Journal of Rehabilitation Medicine, 2009, 24(7): 644—646

Abstract **Objective:** To study the effects of postconditioning on the expression of heat shock protein (HSP) 70 in rats with myocardial ischemia reperfusion. **Method:** The rat′s myocardial ischemia model was established in anesthetized open-chest rat, the left anterior descending (LAD)coronary artery was occluded for 40 min and reperfused for 4h. The total of 48 rats were randomly divided into three groups: ischemia reperfusion control(R) group (n=16): no intervention at reperfusion; ischemic postconditioning (IP)group (n=16): after LAD occlusion, three cycles of 10s reperfusion followed by 10s LAD re-occlusion were applied during the first min of reperfusion; sham operation(S) group(n=16): the surgical procedure was identical to other groups, but the LAD ligature was not ligated. The expression of HSP70 was detected by immunohistochemistry. The presence of apoptotic myocytes was detected by the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method. Meanwhile, the serum creatine kinase activity was measured. **Result:**Serum creatine kinase activity in IP group was lower than that in control group (P<0.05). There was no significant apoptosis after reperfusion in sham-operation group, and the apoptotic rate in IP group was remarkably lower than that in ischemic reperfusion group. The amount of HSP70 in IP group was significant higher than that in control group and sham operation group(P<0.05). **Conclusion:**IP could alleviate reduce myocardial ischemia reperfusion injury significantly. The myocardial protection of postconditioning may be realized by up-regulating the expressions of HSP70 and decreasing myocardial apoptosis.

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Key words reperfusion injury; heat shock protein; apoptosis

缺血后处理是指心肌发生缺血后, 开始再灌注时进行数次短暂再灌注/缺血循环的处理方式,它是近年提出的一种新的减轻再灌注损伤的方法, 研究表明缺血后处理可以缩小心肌梗死面积, 减少再灌注心律失常,具有心肌保护作用[1—4]。热休克蛋白在心肌缺血再灌注中起着重要的保护作用[5],缺血后处理对热休克蛋白表达的影响尚未见研究报道, 本文旨在探讨在体情况下缺血后处理对大鼠缺血/再灌注心肌热休克蛋白表达的影响。

1 材料与方法

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1.1 实验动物

健康清洁级 SD 大鼠 48 只,雄性,体质量(250±30)g,由北京大学医学部实验动物中心提供。

1.2 动物模型制备及分组

1.2.1 动物模型制备:实验大鼠以 30g/L 戊巴比妥钠(45mg/kg)腹腔注射麻醉,仰卧固定于鼠台,行气管切开,用呼吸机进行机械通气,通气频率 60 次/min,潮气量 20—30ml/kg,取左 T3 肋间进胸,暴露心脏,于左心耳根部下方 1mm 处进针,5—0 丝线(结扎线)穿过心肌表层在肺动脉圆锥处稍下方穿线,线两端各穿一小缝合垫片后再并线一起经内径约 2mm 的聚乙烯管中穿出,用血管钳推压小管压迫左冠状动脉前降支造成缺血,放松即可恢复血流形成再灌注,收紧结扎线心电图出现 ST 段抬高,放松结扎线 ST 段下降 1/2 以上为模型成功。

1.2.2 动物分组:随机分为 3 组:①假手术组(sham),开胸后穿线做套环,但不收紧结扎线;②缺血再灌注组(control),收紧结扎线缺血 40min,放松结扎线再灌注 240min;③缺血后处理组 (IP),缺血 40min 后,再灌注 10s,缺血 10s,连续 3 个循环,然后再灌注 240min。

1.3 检测指标

1.3.1 CK 的检测:再灌注结束后自右颈动脉分别采血 2ml,3000r/min 离心 10min,−70 ℃冰箱保存待测。采用 7180 型全自动生化分析仪(日本日立公司)检测 CK 的含量,参照试剂盒(北京中山生物技术有限公司)说明书操作;

1.3.2 心肌 HSP70 分析:再灌注结束后,将缺血区心肌组织剪下,40g/L 多聚甲醛固定 24h,常规石蜡包埋,免疫组织化学检测 HSP70 的表达。(HSP70 单克隆一抗为美国 Sigma 生物试剂公司生产)心肌细胞浆呈棕黄色者为阳性,每个标本取 1 张切片,每张切片随机选 5 个视野(×400),每个视野随机选 5 个区域,用 M BioMias 图像分析系统测每个区域的吸光度 (absorbency, A),计算 25 个区域的平均吸光度。

1.3.3 凋亡细胞原位标记与凋亡指数计算:再灌注结束后,将缺血区心肌组织剪下,40g/L 多聚甲醛固定 24h,常规石蜡包埋,用 TUNEL 技术标记凋亡的细胞核,二脒基苯基吲哚衬染。在免疫荧光显微镜下观察心肌细胞凋亡情况,右紫外激发光下,所有细胞核发出蓝色荧光;在蓝色激发光下,凋亡心肌细胞核发出绿色荧光,每个标本选取 5 张切片,每张切片选取 5 个视野(×400),统计细胞总数和凋亡细胞数,以平均阳性细胞数所占的百分比作为凋亡指数 (细胞凋亡原位检测试剂盒购自 Roche 公司)。

1.4 统计学分析

采用 SPSS 11.5 进行数据处理,计量数据用均数±标准差表示,两组间比较采用 t 检验,多组间比较采用方差分析,$P<0.05$ 为差异有显著性意义。

2 结果

2.1 血清肌酸激酶活性测定

再灌注结束后缺血后处理组和缺血再灌注组肌酸激酶活性明显高于假手术组,依次为 (712.13±42.77),(935.17±57.99),(282.74±29.54)U/L,$P<0.05$,缺血后处理组明显低于对照组($P<0.05$)。

2.2 心肌凋亡细胞计数

再灌注结束后假手术组未见明显细胞凋亡(<5%),缺血后处理组心肌细胞凋亡率明显低于缺血再灌注组,依次为 14.3%±2.7%,22.3%±3.6%($P<0.05$)。

2.3 心肌 HSP70 表达

缺血后处理组较对照组 HSP70 吸光度高(分别为 32.17±13.26,6.83±2.14,$P<0.05$),见图 1(见彩色插页)。

3 讨论

再灌注损伤问题自提出以来一直未能很好地解决,缺血预适应虽然能减轻再灌注损伤,但由于其需要在心肌缺血前实施,因而临床应用受到限制。许多药物、电针动物实验可减轻再灌注损伤[6],但临床应用尚需进一步研究。缺血后处理是近年提出的一种新的减轻再灌注损伤的方法,2003 年,赵志清首先发现缺血后处理对再灌注心肌具有保护作用,随后在多种动物多个实验室得到验证。2005 年,Staat 等[3]率先在临床上证实了缺血后处理的心肌保护作用。其后又有临床研究[7—10],表明缺血后处理能够改善急心肌梗死患者 PCI 后的心肌灌注,缩小心梗面积,改善心脏功能,并具有远期保护作用。

缺血后处理对心肌的保护机制复杂,Zhao 等[1]在犬的在体缺血后处理中证实,缺血后处理可降低血中丙二醛(MDA)含量,减少心肌缺血区超氧阴离子的产生。Kin 等[11]在大鼠模型、Lauzier 等[12]在小鼠模型中也得出了同样的结论,提示缺血后处理可能是通过减少氧自由基的产生而对心肌起到保护作用。Argaud 等研究[13—16]发现缺血后处理可抑制缺血区心肌(MPTP)的开放,缩小心肌梗死面积,缺血后给予 NIM811(MPTP 特异阻断剂)具有和缺血后处理相似的结果,表明缺血后处理可抑制 MPTP 开放

而产生心肌保护作用。ATP 敏感性钾通道(kATP)亦是缺血后处理的保护机制之一，给予其抑制剂可以抵消缺血后处理的心肌保护作用[17—19]。另外再灌注损伤挽救激酶、阿片受体、腺苷受体在缺血后处理心肌保护中起着重要作用[20—23]。

热休克蛋白是机体在多种损伤性应激原作用下合成的一组结构高度保守的、具有保护组织和细胞应激性损伤的内源性蛋白质，在心肌缺血再灌注损伤中起着重要保护作用，其心肌保护机制可能为分子伴侣功能、维护心肌 Ca^{2+}的稳定、保护心肌线粒体功能、抑制凋亡等。近年来,HSP 在参与凋亡的调节上日益受到关注。研究发现,HSP70 可以阻止热应激所致的细胞色素 C 从线粒体的释放,并能与 Apaf-1 结合,抑制 Apaf-1 和 Caspase-9 的复合体的形成,从而抑制凋亡[24—25]。细胞凋亡是心肌缺血/再灌注过程中心肌细胞死亡的重要形式，抑制凋亡可以缩小心肌梗死面积,研究报道[15,26]缺血后处理对缺血再灌注心肌具有抑制凋亡作用，其机制可能与减少氧自由基的生成、抑制缺血区心肌线粒体通透性转运孔道的开放有关，缺血后处理对热休克蛋白表达有无影响，能否增强热休克蛋白表达抑制凋亡尚未见研究报道。本研究发现后处理可增强热休克蛋白表达、减少心肌细胞凋亡,这可能为其心肌保护机制之一,为临床应用进一步提供了基础研究依据。

本研究表明缺血后处理可减轻缺血再灌注损伤,其机制可能与增强热休克蛋白表达,减少心肌细胞凋亡有关。

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