中南大学学报(医学版) 2010, 35(6) 598- DOI： 10.3969/j.issn.1672-7347.2010. ISSN: 1672-7347 CN: 43-1427/R

论著

注射用心肌肽对幼鼠离体心脏缺血再灌注能量代谢的影响

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摘要：

目的：探讨注射用心肌肽对幼鼠离体心脏缺血再灌注能量代谢的影响及机制。方法：50只SD幼鼠，随机分为5组（n=10），正常对照组：心脏灌流20 min稳定后,再持续灌注150 min；正常+注射用心肌肽组：同正常对照组，但在灌流液中加入注射用心肌肽50 mg/L；模型对照组：心脏灌流20 min稳定后,停搏液停搏（缺血）90 min，再恢复正常灌流（再灌注）60 min，建立心肌缺血再灌注损伤模型，灌流用K-H缓冲液，停搏用ST.Thomas’II停搏液；注射用心肌肽1组(CMP1组)：停搏液加入注射用心肌肽100 mg/L；CMP2组：灌流液加入注射用心肌肽50 mg/L及停搏液加入注射用心肌肽100 mg/L）。监测不同组离体灌流心脏心率(HR）、收缩力(△T)及最大收缩速度(+dT/dtmax)和最大舒张速度(-dT/dtmax)、冠状动脉流量(CF)、复搏时间，透射电镜观察缺血再灌注损伤模型组中幼鼠心肌组织超微结构改变。进一步检测3个缺血再灌注损伤组冠状动脉流出液中肌酸激酶同工酶（CK-MB）含量的变化,心肌组织能量及氧化抗氧化代谢指标、丙二醛、NO的含量，检测心肌组织NOS、醛糖还原酶的活性，实时荧光定量PCR相对定量检测心肌组织iNOS，eNOS和Akr1b4 mRNA表达。结果：正常对照组离体心脏长时间灌流后心功能无明显变化，注射用心肌肽对正常心脏心功能无明显影响。与模型对照组比较，两个用药组心搏功能下降轻微，冠状动脉流量较好。电镜观察可见模型对照组心肌肌原纤维断裂，线粒体肿胀，而用药组心肌组织结构较完整，线粒体未见明显肿胀变性。再灌注后，模型对照组CK-MB增加。与模型对照组比较， 两个用药组的CK-MB含量低，Na+-K+ ATP酶、Ca2+-Mg2+ ATP酶、心肌总ATP酶活性相对较高，超氧化物歧化酶活性也增高，丙二醛、NO含量低，心肌组织NOS、醛糖还原酶的活性下降，iNOS，Akr1b4 mRNA表达下调，其中CMP2组改变更为明显（P＜0.01或P＜0.05）。此外eNOS mRNA表达在CMP2组升高（P＜0.05），而在CMP1组中变化无统计学意义（P>0.05）。结论： 注射用心肌肽可改善缺血心脏再灌注后的能量代谢，增加冠状动脉流量，提高心功能，对幼鼠未成熟心肌缺血再灌注损伤具有保护作用，灌流液及停搏液均加入注射用心肌肽对未成熟心肌缺血再灌注损伤的保护作用效果更佳。其作用机制可能与抑制心肌细胞iNOS, Akr1b4 mRNA表达，减少NO生成，抑制醛糖还原酶的活性有关。

关键词： 心肌肽；再灌注损伤；NO；NOS；醛糖还原酶

Effect of cardiomyopeptidin for injection on energy metabolism in isolated hearts of young rats after ischemia-reperfusion injury

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Abstract:

ObjectiveTo investigate the effect of cardiomyopeptidin for injection on energy metabolism in isolated hearts of young rats after ischemia-reperfusion injury. MethodsFifty young healthy SD rats（aged 20±3 days and weighing 50-70 g）were randomly divided into 5 groups: a normal control group (NC group, n=10): the isolated hearts were stable for 20 min, and then 150 min continuous perfusion; a normal + cardiomyopeptidin group (NCMP group, n=10): the same as the normal control group, but K-H buffer solution was added with 50 mg/L cardiomyopeptidin, and 3 ischemia-reperfusion injury model groups, including a model control group (n=10): the isolated rat hearts were perfused with K-H buffer and then arrested with cardioplegic solution; a CMP1 group (n=10): the ST.Thomas’II cardioplegic solution was added with 100 mg/L cardiomyopeptidin; CMP2 group (n=10): K-H buffer and ST.Thomas’II cardioplegic solution was added with 50 mg/L and 100 mg/L cardiomyopeptidin respectively. The cardiac functional indexes were monitored, including heart rate, myocardial contractility and diastolic function, peak systolic and diastole myocardial velocities and coronary flow. In the 3 ischemia-reperfusion injury model groups, myocardial ultrastructure was observed through transmission electron microscopy; the creatine kinase isoenzyme (CK-MB) concentration was measured in the fluid outflow of coronary; the content of Na+-K+ ATPase, Ca2+-Mg2+ ATPase, total ATPase, superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide(NO), total nitric oxide synthase (TNOS), inducible nitric oxide synthase (iNOS) and aldosereductase were measured in the myocardium tissue; the relative expression levels of iNOS, eNOS, and Akr1b4 mRNA in the myocardial tissue were also detected by real-time

fluorescence quantitative PCR. ResultsIn the NC group, after prolonged perfusion, the cardiac function of isolated hearts had no significant change. Cardiomyopeptidin for injection had no significant effect on normal isolated hearts. Compared with the model control group, the cardiac function indexes and coronary flow in the groups treated with cardiomyopeptidin decreased much less. Cardiac myofibrillar fragmentation and mitochondrial swelling were observed in the control group, while in the CMP groups, the myocardial structure was nearly complete, and only mild mitochondria swelling and degeneration could be seen. After the reperfusion, the content of CK-MB was increased in the control group. Compared with the model control group, the CK-MB content was lower in the CMP1 and CMP2 groups. There was a slight decline in the contents of Na+-K+ ATPase, Ca2+-Mg2+ ATPase, and Total ATPase in the CMP1 and CMP2 groups, and an increase in SOD activity ($P<0.01$ or $P<0.05$). The concentration of NO and MDA produced after the ischemia-reperfusion injury was much lower in the CMP1 and CMP2 groups. The activity of iNOS and aldosereductase was inhibited, the expression levels of iNOS, and Akr1b4 mRNA were significantly down-regulated in the CMP1 and CMP2 groups. These changes were more prominent in the CMP2 group ($P<0.01$ or $P<0.05$). The eNOS mRNA levels in the CMP2 group was up-regulated ($P<0.05$). ConclusionCardiomyopeptidin for injection may improve the energy metabolism, improve coronary blood flow and car-diac function after the reperfusion, thus protecting immature myocardial against ischemia-reperfusion injury in young rats. Administration of it in both K-H buffer and ST.Thomas' II cardioplegic solution is better than adding it in cardioplegic solution alone. The mechanism may be associated with the inhibition the mRNA expression of iNOS and Akr1b4 in cardiomyocytes, the inhibition activity of iNOS and aldosereductase, and the decrease of NO production.

Keywords: cardiomyopeptidin;ischemia-reperfusion injury;nitric oxide;nitric oxide synthase;aldosereductase

收稿日期 2009-10-12 修回日期 网络版发布日期

DOI: 10.3969/j.issn.1672-7347.2010.

基金项目:

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