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超氧化物歧化酶对离体大鼠工作心脏和培养心肌细胞缺血再灌注损伤的作用

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Effects of superoxide dismutase on ischemic reperfusion injury in isolated working heart and cultured myocardial cells of rats

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ABSTRACT An isolated working rat heart underwent 40 min of normothermic ceasing perfusion and 25 min of reperfusion. Superoxide dismutase (SOD) or / and mannitol were added to the perfusate 15 min before ceasing perfusion and 15 min after reperfusion. The results indicated that SOD (37 000 IU / L) improved significantly the contractile function of heart and increased the aortic output and coronary flow. Mannitol 0.02 mol / L provided additional benefit. The rat myocardial cells were cultured in the medium for 3 h with hypoxia followed by 2 h of reoxygenation. The content of lactic dehydrogenase (LDH) in the medium was increased and the degree of fluorescence polarization of myocardial cell membrane was raised. SOD was effective in preventing LDH release and decreasing the degree of fluorescence polarization. These results clearly demonstrate that ischemic reperfusion are capable of causing significant myocardial injury, which can be reduced or prevented by administration of oxygen free radical scavenger SOD.

KEY WORDS superoxide dismutase; myocardial reperfusion injury; heart; cultured cells; membrane fluidity; cell membrane permeability

提要 离体大鼠工作心脏 37 ℃停灌 40 min 后再灌注，心脏功能明显降低，灌流液中加入超氧化物歧化酶(SOD) 37 000 IU / L 有明显保护作用，甘露醇 0.02 mol / L 对 SOD 具有协同作用. 在培养基中加入 SOD 可减少无氧无糖再给氧给糖培养大鼠心肌细胞释放 LDH，并防止心肌细胞膜流动性降低.

关键词 超氧化物歧化酶; 心肌再灌注损伤; 心脏; 培养的细胞; 膜流动性; 细胞膜通透性

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超氧化物歧化酶(superoxide dismutase, SOD)是一种重要的氧自由基清除剂. 国外用整体动物或 Langendorff 法灌流离体心脏等研究 SOD 对心脏缺血再灌注损伤的影响证明: SOD 具有缩小心肌梗塞范围[1]、改善心脏功能[2]、清除心律失常[3]、减轻病理改变[2]等作用. 但未见 SOD 对离体工作心脏及培养心肌细胞作用的报道. 离体工作心脏既消除了神经体液因素对心脏的影响，又保留了心脏的正常泵血功能，有利于观察 SOD 对心脏的作用; 直接测定培养心肌细胞释放乳酸脱氢酶(lactic dehydrogenase, LDH)及心肌细胞膜流动性对阐明 SOD 的作用机理有所俾益.

MATERIAL AND METHODS

SOD (Cu · Zn · SOD): 盐城生物制药厂提供，苏州医学院监制，淡蓝色无嗅粉末，易溶于水，分别以灌流液或培养液配制. 甘露醇: 分析纯，上海试剂二厂出品. Eagle 培养基: 日本产品; 无糖 Eagle 培养基: 中国科学院生物物理所生物化学试剂厂生产; DPH(1,6-二苯基-1,3,5-己三烯):Sigma 产品.

离体大鼠工作心脏 Wistar 大鼠，♂，36 只，按 Flynn 等[4,5]方法制备工作心脏，稳定后持续灌流 10 min，观测下列指标并通过不同换能器连至 SJ-42 生理记录仪同步描记: 主动脉内收缩压(AOP)及其微分($\pm dP/dt$); 左心室内压(LVP)及其微分($\pm dP/dt$); 心电图 II 导(ECG); 主动脉输出量(AF)及冠脉流量(CF)经流量记录仪记录. 实验程序及分组: 每 5 min 记录上述指标一次，连续记录两次后制备缺血再灌注模型: 改顺灌为逆灌 15 min，继之 37℃停灌 40 min，再灌注 25min，其中开始 15 min 为逆灌，后 10 min 为顺灌，停灌前 15 min

给以含药灌流直至再灌注后 15 min,共分 4 组: A 组(n=9): 灌流液不含任何药物; B 组(n=12): 灌流液中加入 SOD, 终浓度 37 000 IU / L; C 组(n=8): 灌流液含 SOD 37 000 IU / L 和甘露醇 0.02 mol / L; D 组(n=7): 灌流液含甘露醇 0.02 mol / L.

培养大鼠心肌细胞 Wistar 乳鼠, 辛 3 不拘, 出生 1–4 d, 按我室沿用方法[6]制备培养心肌细胞, 用 0.1% 胰蛋白酶溶液分次消化心室肌细胞, 所得细胞用含 20% 小牛血清的 MEM Eagle's 液稀释成 1×10^6cells / ml, 接种于培养瓶中, 37℃ 密闭培养. 2–3 d 后进行无氧无糖及再给氧给糖培养[7]. 细胞分 4 组: 每组 8 瓶, 第 1 组为正常培养组; 第 2 组为无氧无糖及再给氧给糖培养模型组: 无氧无糖培养 3h 后再给氧给糖培养 2h; 第 3 组为 SOD 小剂量组: 培养液中加入 SOD 370 000 / IU / L; 第 4 组为 SOD 大剂量组: 培养液中加入 SOD 740 000 IU / L.

培养心肌细胞释放 LDH 测定[8]: 每培养瓶为一样本, 分别留取无氧无糖及再给氧给糖培养液 0.1ml, 检测在辅酶 I 存在时, LDH 使乳酸钠转化成丙酮酸的含量, 间接测定 LDH 活性.

心肌细胞膜流动性测定[9]: 经无氧无糖及再给氧给糖培养的心肌细胞以 DPH 37℃ 标记细胞膜脂质 30 min, 在 RF-510 荧光分光光度计上以激发波长 λ_{ex}=363 nm, 发射波长 λ_{em}=428 nm 分别测定起偏器和检偏器处于垂直和水平 4 个不同方向的荧光读数. 并计算荧光偏振度, 以此了解膜流动性.

RESULTS

SOD 对离体大鼠工作心脏停灌再灌注损伤的影响 离体大鼠工作心脏 37℃ 停灌 40 min 后恢复正常灌注. 在再灌注开始时, 9 只中 4 只持续室颤, 1 只停搏长达 10 min, 再灌注 15 min 后, 心率减慢、主动脉输出量和冠脉流量减少, 左心室收缩内压, 主动脉内压降低 (Tab 1).

Tab 1. Effect of superoxide dismutase (SOD 37 000 IU / L) on arrhythmias after ischemic reperfusion in isolated rat hearts. $\bar{x}\pm$ SD. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

	n	Incidence of VF		Continuing time of arrhythmias (min)
		Positive	Negative	
Control	9	4	5	11.4±7.9
SOD	12	2**	10**	3.5±3.5**
SOD+Man	8	0*	8**	1.4±0.5***
Man	7	2*	5*	8.1±5.8*

Man : mannitol 0.02 mol / L; VF: ventricular fibrillation.

SOD 可明显缩短再灌注心律失常持续时间,促进冠脉流量和主动脉输出量恢复,然而, SOD 及甘露醇对左心室收缩压及主动脉收缩压的下降, 室颤的发生无明显防止作用. 若 SOD 与甘露醇合用, 可明显降低室颤发生率, 防止左心室内压下降, 以致与停灌前无明显差异($P>0.05$), 延缓主动脉内压下降, 并加速其恢复(Tab 2).

SOD 对无氧无糖再给氧给糖培养大鼠心肌细胞的影响 心肌细胞释放 LDH 心肌细胞无氧无糖培养 3 h 释放的 LDH 量明显增加, 而且在再给氧给糖培养 2 h 释放量仍较正常为高. 在培养基中加入 SOD 740 000 IU / L 可使心肌细胞释放 LDH 减少, 并接近正常.

心肌细胞膜流动性 经无氧无糖再给氧给糖培养的心肌细胞膜荧光偏振度明显高于正常培养心肌细胞, 可见细胞膜流动性降低, 在培养基中加入 SOD 370 000 IU / L 或 740 000 IU / L 可预防其降低(Fig 1).

DISCUSSION

本文结果提示: 离体大鼠工作心脏停灌前 15 min 给予 SOD 37 000 IU / L 并持续至再灌注后 15 min, 可明显减轻停灌再灌注所致心功能改变. 这与许多学者报告[1–3]相符, SOD 的作用在于其清除停灌再灌注时产生的超氧阴离

Tab 2. Cardiac function parameters pre-ischemic and post-ischemic reperfusion in isolated working rat hearts. C: Control, $n=9$; S:SOD 37 000 IU / L, $n=12$; S-M: SOD 37 000 IU / L and mannitol 0.02 mol / L, $n=8$; M: Mannitol 0.02 mol / L, $n=7$. $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* pre-ischemia. $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$ *vs* control. ( ): Recovery rate (%).

Parameter		Pre-ischemia (min)		Reperfusion (min)			
		5	10	5		10	
HR(bpm)	C	172 ± 44	177 ± 44	145 ± 62***	(82 ± 25)	139 ± 52***	(82 ± 34)
	S	197 ± 46	193 ± 44	131 ± 52***	(64 ± 20++)	153 ± 63***	(80 ± 35+)
	S-M	185 ± 24	191 ± 35	146 ± 36***	(79 ± 18+)	135 ± 46***	(71 ± 18+)
	M	196 ± 27	191 ± 24	159 ± 65**	(79 ± 26+)	148 ± 68***	(74 ± 28+)
LVSP	C	6.2 ± 1.2	6.2 ± 1.1	4.9 ± 1.1***	(80 ± 12)	5.0 ± 0.9*	(115 ± 34)
(kPa)	S	5.2 ± 0.9	5.4 ± 0.7	4.5 ± 1.1**	(89 ± 23+)	4.2 ± 0.8***	(79 ± 11+++)
	S-M	7.2 ± 2.3	7.1 ± 2.3	7.5 ± 1.8*	(110 ± 28++)	7.0 ± 2.0*	(108 ± 30+)
	M	6.8 ± 1.5	6.7 ± 1.1	6.2 ± 0.7**	(93 ± 16+)	6.1 ± 0.6**	(92 ± 7+)
$+dP/dt_{max}$	C	58 ± 24	53 ± 22	16 ± 7**	(31 ± 17)	13 ± 8***	(31 ± 22)
(kPa / s)	S	57 ± 14	54 ± 14	26 ± 10***	(44 ± 18+)	24 ± 14***	(44 ± 20++)
	S-M	61 ± 28	60 ± 28	41 ± 35*	(71 ± 45++)	34 ± 30**	(77 ± 66+)
	M	57 ± 28	52 ± 17	19 ± 8***	(35 ± 11+)	21 ± 8***	(37 ± 14+)
$-dP/dt_{max}$	C	34 ± 13	25 ± 12	6 ± 4***	(27 ± 23)	7 ± 1***	(42 ± 24)
(kPa / s)	S	31 ± 9	30 ± 8	14 ± 5***	(48 ± 22++)	10 ± 6***	(35 ± 20+)
	S-M	31 ± 21	28 ± 16	15 ± 14***	(55 ± 31++)	12 ± 8***	(54 ± 32+)
	M	26 ± 7	26 ± 11	12 ± 9***	(45 ± 31+)	11 ± 9***	(43 ± 24+)
LVEDP	C	3.2 ± 1.0	3.2 ± 1.0	3.8 ± 1.3**	(123 ± 27)	4.0 ± 1.3**	(117 ± 36)
(kPa)	S	2.3 ± 0.7	2.4 ± 0.6	2.5 ± 0.8*	(113 ± 19+)	2.8 ± 1.0**	(116 ± 19+)
	S-M	4.2 ± 1.1	4.2 ± 1.1	4.8 ± 1.4**	(120 ± 27+)	4.8 ± 1.4**	(120 ± 24+)
	M	4.1 ± 0.8	3.9 ± 0.7	4.7 ± 0.3**	(116 ± 16+)	4.9 ± 0.3***	(126 ± 23+)
CF	C	2.8 ± 1.4	2.3 ± 0.9	0.7 ± 0.3***	(31 ± 15)	0.5 ± 0.3***	(28 ± 16)
(ml / min)	S	3.2 ± 0.6	3.3 ± 0.6	1.6 ± 0.8***	(47 ± 19++)	1.4 ± 0.6**	(43 ± 12+++)
	S-M	2.4 ± 0.4	2.3 ± 0.5	1.4 ± 0.3***	(60 ± 18+++)	1.4 ± 0.4***	(70 ± 36+++)
	M	3.3 ± 1.0	4.3 ± 2.0	1.4 ± 0.7**	(43 ± 19+)	1.1 ± 1.0***	(32 ± 27+)
AF	C	19.6 ± 4.5	18.3 ± 7.1	8.8 ± 7.1***	(35 ± 24)	3.2 ± 3.5***	(16 ± 18)
(ml / min)	S	21.3 ± 2.5	21.9 ± 5.4	12.1 ± 5.2***	(57 ± 22++)	11.7 ± 8.5***	(56 ± 43***)
	S-M	17.7 ± 2.5	18.0 ± 3.4	9.1 ± 6.1***	(61 ± 32++)	8.9 ± 6.7***	(52 ± 40++)
	M	19.7 ± 1.7	20.0 ± 4.0	7.9 ± 6.2***	(39 ± 30+)	4.9 ± 4.7***	(23 ± 22+)
AOP	C	9.5 ± 1.7	9.2 ± 1.6	6.5 ± 1.3***	(70 ± 14)	6.4 ± 1.4***	(73 ± 12)
(kPa)	S	9.1 ± 1.0	9.4 ± 0.7	7.3 ± 1.6***	(82 ± 21+)	6.5 ± 1.1***	(69 ± 8+)
	S-M	9.4 ± 2.3	9.7 ± 2.2	8.4 ± 1.7*	(94 ± 16+++)	8.3 ± 2.0***	(89 ± 6+)
	M	10.1 ± 1.9	10.1 ± 1.6	8.6 ± 1.0**	(83 ± 9+)	8.6 ± 1.1***	(83 ± 8+)
$+dP/dt_{max}$	C	108 ± 25	99 ± 29	36 ± 14***	(32 ± 17)	29 ± 29***	(27 ± 23)
(kPa / s)	S	119 ± 30	129 ± 22	87 ± 28**	(75 ± 23+++)	71 ± 30**	(55 ± 22+++)
	S-M	95 ± 27	98 ± 23	64 ± 39**	(61 ± 27+++)	59 ± 44**	(59 ± 34++)
	M	87 ± 32	78 ± 41	32 ± 11***	(28 ± 26+)	30 ± 12**	(27 ± 25+)
$-dP/dt_{max}$	C	107 ± 29	91 ± 30	27 ± 24***	(30 ± 26)	23 ± 25***	(24 ± 22)
(kPa / s)	S	113 ± 38	121 ± 32	67 ± 28***	(63 ± 28++)	57 ± 35***	(45 ± 28++)
	S-M	100 ± 38	100 ± 36	52 ± 39***	(49 ± 29+)	53 ± 48***	(48 ± 35+)
	M	76 ± 19	67 ± 24	26 ± 22***	(32 ± 24+)	22 ± 20***	(29 ± 26+)

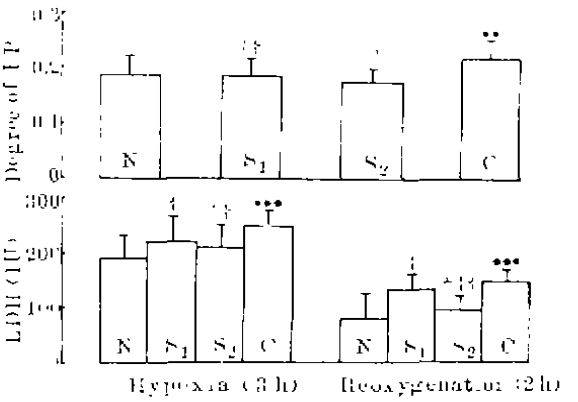

Fig 1. Effects of SOD on lactic dehydrogenase (LDH) release (Lower) and membrane fluidity (Upper) of myocardial cells cultured by hypoxia 3 h and reoxygenation 2 h. N = normal; C = control; S_1 = SOD 370 000 IU / L; S_2 = SOD 740 000 IU / L; FP = fluorescence polarization. $n = 8$, $\bar{x} \pm SD$. $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* N; $^{+}P > 0.05$, $^{++}P < 0.05$, $^{+++}P < 0.01$ *vs* C.

子自由基，从而减轻或消除自由基对心肌的毒性作用[10].

SOD 亦可防止心肌细胞经无氧无糖再给氧给糖培养所致细胞膜通透性增加及膜流动性降低. 而且，SOD 不单在再给氧给糖时发挥作用，对无氧无糖培养心肌细胞亦有作用. 可见氧自由基不仅在再灌注开始时大量增加，且在缺血时已有增加. 因此，须在再灌注前甚至缺血时给予 SOD，其保护作用才能充分显示.

氧自由基性质活泼，可相互转化，亦可直接侵袭细胞膜不饱和脂肪酸，形成脂质过氧化物[11-12]. 质膜脂质过氧化，使细胞膜流动性降低，膜功能难以实现：膜通透性增加，严重时细胞膜结构破坏，使细胞内酶-乳酸脱氢酶等释放，最终导致细胞死亡[13-15].

本实验加用甘露醇(OH · 清除剂) 0.02 mol / L 可增强 SOD 的作用，说明缺血再灌注时不单有 O_2^- 形成增加，且有 OH · 形成加速，后者在缺血再灌注损伤中起一定作用.

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地塞米松对培养大鼠搏动心肌细胞感染 Coxsackie B-2 病毒作用的电生理观察

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Influence of dexamethasone on electrical activities in cultured rat beating myocardial cells infected with Coxsackie B-2 virus

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ABSTRACT The effects of dexamethasone (Dex) on electrical activities in cultured rat beating myocardial cells infected with 100 TCID-50 Coxsackie B-2 virus (CB_2V) was evaluated by conventional intracellular microelectrode technique. The frequency began to increase, the beating % decreased, and multiform arrhythmias were shown in the infected group 24 h post-challenge. Meanwhile, the cytopathic effect (CPE) appeared rapidly from 1+ to 3+. In the infected and Dex-treated group, the beating % was higher and the arrhythmias and CPE were less than in the infected group at the same intervals. The numbers of non-beating cells increased parallel to the incubation time in the infected group. Decreases of maximal diastolic potential (MDP), maximal upstroke rate (V_{max}), overshoot (OS) and action potential amplitude (APA), and abbreviation of action potential duration (APD_{50} and APD_{100}) in infected and Dex-treated group were less than those in control group during 24-96 h post-challenge. Premature beats, tachycardia, bradycardia and fibrillation occurred in the early stages after infection. It is surmised that steroids can probably save the lives of patients with severe myocarditis if Dex was supplemented.

KEY WORDS dexamethasone; cultured cells; Coxsackie B viruses: action potentials; myocardium

提要 Coxsackie B-2 病毒感染培养搏动心肌细胞加地塞米松后 24-96 h 能使感染心肌细胞停搏%, 节律, 最大舒张电位负值, 动作电位振幅和超射, 最大上升速率, 动作电位时程等参数异常大部分有所改善, 提示重症病毒性心肌炎患者宜在发病早期应用较大剂量肾上腺皮质激素, 结合一般支持疗法, 可能有挽救生命的意义.

关键词 地塞米松; 培养细胞; 柯萨奇 B 病毒; 动作电位; 心肌

重症心律失常常为急性病毒性心肌炎的猝死因素, 对于这类患者是否使用肾上腺皮质激素的问题, 临床上至今存有不同的看法[1,2]. 实验室研究也未能得出一致的结论[3]. 我们曾发现地塞米松 (dexamethasone, Dex)能使 Coxsackie B-2 病毒(CB_2V)感染培养搏动大鼠心肌细胞的搏动%增多, 细胞病变(CPE)及

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