研究报告

沙漠干热环境下中暑大鼠的心肌酶及心肌组织形态学改变

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【摘要】 **目的** 探讨沙漠干热环境下中暑对大鼠心肌损伤的影响。**方法** 48 只雄性 SD 大鼠随机平均分成 6 组:沙漠干热环境轻度中暑组及其常温对照组,中度中暑组及其常温对照组,重度中暑组及其常温对照组,然后将 3 个实验组大鼠分别置于干热环境(温度:41℃,湿度:10%),3 个对照组大鼠置于常温环境(温度:25℃,湿度 35%)中,在建立沙漠干热环境中暑大鼠后,干热环境组及其对照组分别在实验开始 70 min(轻度中暑)、110 min(中度中暑)、145 min(重度中暑)被处死并取材。用全自动生化检测仪检测大鼠血清心肌酶 CK(磷酸肌酸激酶)、CK-MB(肌酸激酶同工酶)、LDH(乳酸脱氢酶)的变化,用 HE 染色观察心肌病理学变化,用电子显微镜观察心肌超微结构变化。**结果** 干热中暑各组大鼠血清心肌酶 CK、CK-MB、LDH 较常温对照各组显著升高($P<0.05$),干热组 CK、CK-MB、LDH 均随中暑程度加重而升高,其中 CK 和 LDH 的轻度中暑组与中度中暑或重度中暑组比较差异具有显著性($P<0.05$),但中度中暑组与重中暑度组比较差异无显著性($P>0.05$);干热中暑组 CK-MB 各个组间比较差异均有显著性($P<0.05$);HE 染色结果提示:干热中暑组心肌组织早期即心肌间血管明显出现扩张充血、出血,随热暴露时间延长充血、出血现象逐渐加重,常温对照组未见异常。电子显微镜结果显示:干热环境组心肌细胞部分肌丝紊乱断裂、溶解,Z 线模糊消失,线粒体肿胀,线粒体基膜不清晰,见空泡形成,部分毛细血管内皮细胞增生,并随热暴露时间延长,心肌细胞损伤逐渐加重。**结论** 沙漠干热环境可造成大鼠心肌损伤,并随热暴露时间的延长及中暑程度的加重而损伤逐渐加重。提示沙漠干热环境下中暑的治疗应注意加强心肌损伤的保护。

【关键词】 沙漠; 干热环境; 中暑; 心肌酶; 心肌损伤

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Changes of myocardial enzymes, histology and ultrastructure of heat stroke rats in dry-heat desert environment

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【Abstract】 **Objective** To explore the myocardial enzyme, histology and ultrastructure changes of heat stroke rats in dry-heat desert environment. **Methods** Forty-eight male SD rats were randomly divided into 6 groups: mild heat stroke group and its control group, moderate heat stroke group and its control group, severe heat stroke group and its control group. Then the three experimental groups of rats were put into dry-heat environment (temperature 41℃, humidity 10%) and the three control groups were put into normal environment (temperature 25℃, humidity 35%). After establishment of

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the heat stroke rat models, the rats were sacrificed at their corresponding time points (70, 110 and 145 min) from the beginning of the experiment for the mild heat stroke group and its control group, moderate heat stroke group and its control group, and severe heat stroke group and its control group, respectively. Blood samples were taken and heart tissues were harvested. The serum enzymes CK, CK-MB, and LDH were detected by an automatic biochemical analyzer. The pathological examination was performed with HE staining and ultrastructural changes were observed by electron microscopy. **Results** The serum enzymes CK, CK-MB, LDH were significantly higher in the dry-heat stroke groups than that in their control groups ($P<0.05$). The serum CK, CK-MB, LDH levels were increased along with the progression of heat stroke, of which, CK and LDH of the mild heat stroke group were significantly different compared with that of the moderate heat stroke group or severe heat stroke group ($P<0.05$). However, there were no significant difference between the moderate and severe heat stroke groups ($P>0.05$). CK-MB levels were significantly different between every two groups of the three heat stroke groups ($P<0.05$). The pathological examination showed dilation and congestion of blood vessels and hemorrhage, which became more serious along with the prolongation of exposure to dry-heat. The control group showed no abnormalities. Electron microscopy showed disruption of myofilaments and myolysis, blurred Z lines, swollen mitochondria, cytoplasmic vacuolization in the cardiomyocytes of heat stroke rats, and all these myocardial cell injuries became more serious along with the progression of heat-stroke. **Conclusions** Dry-heat desert environment can cause myocardial injury, and gradually getting worse along with the prolongation of dry-heat exposure and progression of heat stroke. Our findings suggest that attention should be paid to protection of the myocardium against injurious effect of heat stroke in dry-heat desert environment.

【**Key words**】 Desert; Dry-heat environment; Heat stroke; Myocardial enzyme; Myocardial injury; Pathology; Ultrastructure; Rats

沙漠干热环境以高温、干燥、缺水少雨等为主要特点,该环境下热损伤容易使机体发生中暑,中暑是热损伤的严重表现,是一种威胁生命的严重疾病,临床上定义为核心体温超过 40℃,机体温度调节失败,中枢神经系统异常出现抽搐、昏迷、谵妄等症状[1]。,高热可引起全身炎症反应导致多器官功能障碍或衰竭,包括在大脑、肺、心脏、胃肠道、肾的出血和坏死[2,3]。在高温环境中,即使是很短的热暴露会导致机体死亡[4],这种快速导致机体死亡的原因可能与某个器官脏器的损伤直相有关。目前少有沙漠干热环境中暑对机体损伤的文献报道,本研究在我院西北特殊环境人工实验舱内建立沙漠干热环境大鼠中暑模型,探讨不同中暑阶段对大鼠心肌的损伤性变化,为在沙漠干热环境中暑的救治过程中心肌的保护提供理论依据。

1 材料和方法

1.1 实验动分组及材料

根据我们先前的实验,在“西北特殊环境人工实验舱”内建立沙漠干热环境中暑模型,将 SPF 级健康雄性 SD 大鼠 48 只(合格证号:SCXK(新)2011-0001)随机分成 6 组,每组 8 只:沙漠干热环境轻度中暑组及其常温对照组,中度中暑组及其常温对照组,重度中暑组及其对照组。实验开始,每只大鼠用 3% 戊巴比妥按 40 mL/kg 行腹腔内麻醉,麻醉生效后被置于仰卧位,大鼠的右侧股动脉被分离并插入一静脉留置针(24G)。三个电极被连接到大鼠的肢体上,一根精密玻璃水银温度计被插入于大鼠的肛门中。然后,三组中暑组的大鼠均通过压力传感器、心电传感器与多通道生理记录仪连接起来(泰盟 BL420F,中国),并被移置于模拟沙漠干热环境舱内(西北特殊环境人工实验舱,兰州军区乌鲁木齐总医院),设定温度 41℃,湿度 10%。而 3 组对照组的大鼠仍被置于常温环境(温度 25℃,湿度 35%)中。血清心肌酶检测及心肌 HE 病理分析由兰州军区乌鲁木齐总医院检验科及病理科检测分析,心脏标本送新疆医科大学用透射电子显微镜观察心肌细胞结构,拍照并进行分析。

1.2 取材及检测项目

(1)血清心肌酶:大鼠腹腔麻醉后处死,留置针抽取下腔静脉血液,促凝管保存,3 500 r/min 离心 15 min, −80℃保存,全自动生化检测仪检测血清磷酸肌酸激酶(CK)、CK 同工酶(CK-MB)、乳酸脱氢酶(LDH)水平。(2)HE 染色:大鼠处死后,分离并切取适当大小心脏组织,生理盐水冲洗后放入新鲜配制 4% 多聚甲醛中固定,进行石蜡包埋和切片,切片厚约 4 mm,苏木素一伊红(HE)染色后观察心脏病理变化。(3)电镜:切取心脏心尖部大小约 1.5

mm × 1.5 mm 大小心脏组织,立即放入新鲜配制的 2.5% 的戊二醛中,将固定后的心肌组织送新疆医科大学,经四氧化锇固定、梯度脱水、包埋及聚合后,做超薄切片,经醋酸双氧铀、枸橼酸铅双重染色各 10 min,用高倍投射电子显微镜观察心肌结构,拍照并进行分析。

1.3 统计学方法

用 SSPS 17.0 软件分析,计量资料采用均数 ± 标准差($\bar{x} \pm s$)表示,组间比较应用单因素方差分析,$P < 0.05$ 为差异有统计学意义。

2 结果

2.1 血清心肌酶的变化

干热中暑各组大鼠血清心肌酶 CK、CK-MB、LDH 明显较常温对照各组升高($P < 0.05$),干热组 CK、CK-MB、LDH 均随中暑程度加重而升高,其中 CK 和 LDH 的轻度中暑组与中度中暑组或重度中暑组比较差异具有显著性($P < 0.05$),但中度中暑组与重度组比较差异无显著性($P > 0.05$);干热中暑组 CK-MB 各个组间比较差异均有显著性($P < 0.05$)。见表 1。

2.2 HE 染色光镜观察

常温对照组心肌肌束排列整齐,心肌细胞未见水肿,心肌肌间隙血管未见扩张及出血(图 1,2,3),干热中暑组光镜下心肌组织早期即心肌间血管明显出现扩张充血、出血,随热暴露时间延长充血、出血现象逐渐加重(图 4,5,6)(图 1 ~6 见彩插 1)。

2.3 电镜观察心肌细胞超微结构

常温对照组,心肌细胞形态完整,心肌纤维排列整齐,肌节明暗带清晰,线粒体排列整齐,线粒体膜完整(图 7,8,9)。干热中暑组心肌细胞部分肌丝紊乱断裂、溶解,Z 线模糊消失,线粒体肿胀,线粒体膜不清晰,见空泡形成,部分毛细血管内皮细胞增生,并随热暴露时间延长心肌细胞损伤逐渐加重(图 10,11,12)。

3 讨论

中暑是一种危及生命的疾病,通过中枢神经系统功能障碍,提升核心体温到 40℃以上,引起全身炎症、血管内凝血和组织病变,可认为是一种全身炎症反应综合征,由于高热引起热耗散导致体表血管扩张,使流入内脏器官的血液减少,特别是肠粘膜的缺血可导致黏膜通透性增加,肠道细菌内毒素可能流入血液中,导致多器官衰竭和死亡[3,5-6]。中暑的发病非常复杂类似于脓毒症,体内许多炎症因子如 HMGB1、TNF-a、IL-1 等参与高热引起的全身炎症反应[7-9]。有报告[10]认为中暑引起并发症包括横纹肌溶解症、弥漫性血管内凝血、急性肾损伤、肝病、癫痫、休克、心律失常、多器官功能障碍综合征(MODS)和意识障碍中,急性肾损伤和弥漫性血管内凝血是影响预后的主要危险因素。

机体在热暴露下心脏属于易激惹器官,高热环境可引起汗腺分泌汗液增多,心脏收缩力增强,心输出量增加,心率加快来增加外周循环满足汗液排放[11],高热对心脏的热损伤可直接对心肌造成损害,有关病理组织学和分子生物学分析,热暴露下心室心肌比心房心肌更容易受损伤[12]。中暑也显示为高细胞因子血症,表现为严重败血症和感染性休克,会导致心脏功能障碍,心室扩张和射血分数降低[13],热暴露可直接诱导高细胞因子血症和心功能障碍,可认为是中暑导致机体死亡的重要机制之一[12]。

表 1 大鼠血清 CK(U/L)、CK-MB(U/L)、LD(U/L)变化($\bar{x} \pm s$)

Tab. 1 Changes of serum CK, CK-MB and LD (U/L) in the rats. ($\bar{x} \pm s$)

指标 Item	轻度中暑实验组 Mild heat stroke group		中度中暑实验组 Moderate heat stroke group		重度中暑实验组 Severe heat stroke group	
	干热中暑组 Dry-heat stroke group	常温对照组 Control group	干热中暑组 Dry heat stroke group	常温对照组 Control group	干热中暑组 Dry heat stroke group	常温对照组 Control group
CK	1289.5 ± 372.3[a,b]	799.1 ± 99.8	2 332.0 ± 775.4[a]	797.9 ± 158.7	2 612.1 ± 1 457.1[a,c]	820.6 ± 134.0
CK-MB	341.5 ± 66.3[a,b]	226.8 ± 31.4	629.0 ± 159.6[a]	250.3 ± 41.7	941.3 ± 344.2[a,b,c]	254.1 ± 43.4
LDH	823.3 ± 383.3[a,b]	761.9 ± 184.4	2 117.3 ± 974.5[a]	776.0 ± 118.0	2 184.3 ± 835.9[a,c]	769.0 ± 128.6

注:a 为干热中暑各组与常温组比较,$P < 0.05$;b 为干热中度中暑组与干热轻度中暑组和干热重度中暑组比较,$P < 0.05$;c 为干热轻度中暑组与干热重度中暑组比较,$P < 0.05$。

Note: The myocardial enzymes CK, CK-MB, LD were significantly higher in the dry-heat stroke groups than that in their control groups ($P < 0.05$). CK, CK-MB, LD were increased with the progression of heat stroke. The CK and LD were significantly higher in the moderate and severe heat stroke groups than that of the mild heat stroke group ($P < 0.05$). There were significant differences between the CK-MB levels of every two groups of the three heat stroke groups ($P < 0.05$ for all).

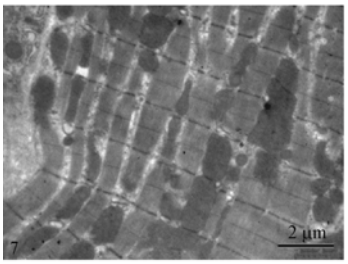

图 7 常温轻度对照组

Fig. 7 A rat of the control group of the mild heat stroke group

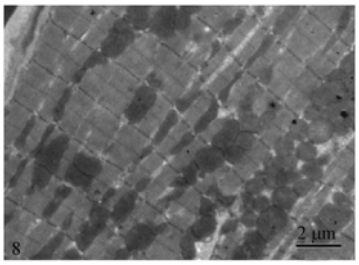

图 8 常温中度对照组

Fig. 8 A rat of the control group of the moderate heat stroke group

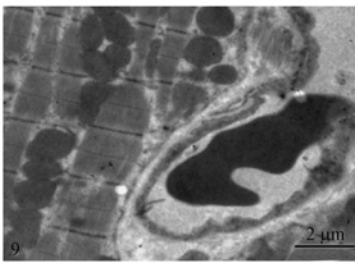

图 9 常温重度对照组

Fig. 9 A rat of the control group of the severe heat stroke group

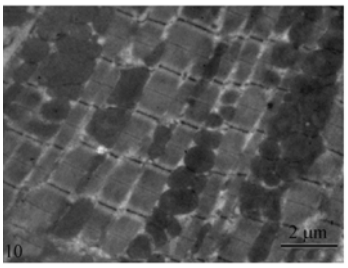

图 10 干热轻度中暑组

Fig. 10 A rat of the mild heat stroke group

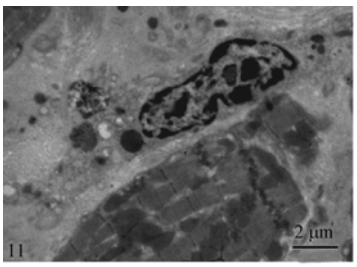

图 11 干热中度中暑组

Fig. 11 A rat of the moderate heat stroke group

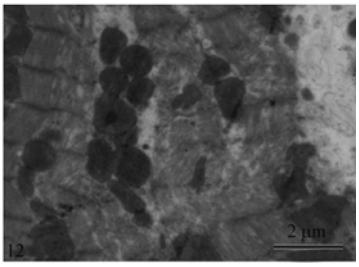

图 12 干热重度中暑组

Fig. 12 A rat of the severe heat stroke group treated in the dry-heat environment

心肌酶 CK、CK-MB、LDH 大量存在于心肌、骨骼肌等组织细胞的胞浆和线粒体中，当心脏病变或受损时，心肌酶表现出不同程度升高，中暑或缺血缺氧时组织细胞受损，这些酶大量释放至血液中，其中 CK 升高最为敏感。本研究中中暑组大鼠心肌酶 CK、CK-MB、LDH 血清学发生明显变化，随热暴露时间延长，中暑程度的加重心肌酶升高水平越高。中暑患者血清 CK 一般认为来自骨骼肌，LDH 的分析，发现 69% 的中暑患者 LDH 水平升高，表明 LDH 来自于心肌组织[14]。Wakino 等[15]报道一个 23 岁中暑患者 4 d 临床过程中表现横纹肌溶解症、急性肾损伤、肝功能衰竭、心肌缺血、因高温造成的心肌需氧量增加、心动过速、心输出量增加，CM-MB 和肌钙蛋白水平明显升高，心电图 ST 段压低和 T 波倒置表现心肌缺血，认为高温与心肌损伤相关。本研究通过病理切片及心肌细胞电镜发现，大鼠在热应激状态下，心肌组织发生明显变化，心肌组织损伤明显，认为血清心肌酶升高与心脏在热应激状态下心肌受损有密切联系。我们应注意中暑可发生心肌损伤[15]。

沙漠干热环境可造成大鼠心肌损伤，并随热暴露时间延长及中暑程度的增加损伤逐渐加重，提示沙漠干热环境下应注意防治心肌损伤的发生。

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