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## 保元煎剂对疲劳大鼠自由基、乳酸、ATP 的影响 \*

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**摘要 目的:**研究保元煎剂对疲劳大鼠力竭游泳时间以及 ATP、羟自由基、乳酸含量的影响。**方法:**选用 60 只雄性 SD 大鼠,随机分为正常组、模型组、西洋参组、保元煎剂低、中、高剂量组。采用力竭游泳加睡眠剥夺建立复合疲劳大鼠模型,均予灌胃给药或蒸馏水(保元煎剂低、中、高剂量依次为  $1.04, 2.08, 4.15 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ;西洋参组给药量为  $0.27 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ),记录大鼠末次力竭游泳时间,检测疲劳大鼠血清 ATP、羟自由基、乳酸含量。**结果:**与模型组比较,保元煎剂各组大鼠末次力竭游泳时间延长,ATP 含量升高,羟自由基及乳酸含量下降,均有统计学意义,  $P < 0.05$ ;与西洋参组比较,保元煎剂高剂量组末次力竭游泳时间延长、乳酸含量降低,保元煎剂高、中、低剂量组 ATP 升高,保元煎剂高、中剂量组自由基水平下降,均具有统计学意义,  $P < 0.05$ 。**结论:**保元煎剂可提高疲劳大鼠力竭游泳时间,升高疲劳大鼠 ATP 含量,降低羟自由基、乳酸含量。

**关键词:**保元煎剂;ATP;羟自由基;乳酸

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## Effects of BaoYuan Decoction on Hydroxyl Radical, Lactic Acid and ATP in Fatigue Rats \*

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**ABSTRACT Objective:** To study the effects of BaoYuan decoction on exhaustion swimming time, the levels of ATP, hydroxyl radical, and lactic acid in fatigue rats. **Methods:** 60 Sprague-Dawley (SD) male rats were randomly divided into normal group, model group, American ginseng group, low-dose BaoYuan decoction group, middle-dose BaoYuan decoction group and high -dose BaoYuan decoction group. Exhaustion swimming with sleep deprivation was adopted to establish the compound fatigue rat model. Then the changes of last exhausted swimming time and ATP, hydroxyl radical and lactic acid in serum after drug or distilled water intervention by gavage (low-dose, middle-dose and high-dose BaoYuan decoction was  $1.04, 2.08, 4.15 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ; American ginseng was  $0.27 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ). **Results:** Compared with model group, the last exhausted swimming time was prolonged, the level of ATP was increased, and the level of hydroxyl radical and lactic acid were reduced in BaoYuan decoction; Compared with American ginseng group, the last exhausted swimming time was prolonged and the content of lactic acid was decreased in high-dose BaoYuan decoction, the level of ATP was increased in all BaoYuan decoction, and the level of hydroxyl radical was reduced in middle-dose and high-dose BaoYuan decoction. All above these were statistically significant ( $P < 0.05$ ). **Conclusions:** BaoYuan decoction can improve exhaustion swimming time of fatigue rats, increase ATP content and reduce the hydroxyl radicals and lactic acid level in fatigue rats.

**Key words:** BaoYuan Decoction; Adenosine Triphosphate; Hydroxyl radical; Lactic Acid

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### 前言

亚健康亦称慢性疲劳综合征 (chronic fatigue syndrome, CFS), 20 世纪 80 年代由前苏联学者 N.布赫曼提出,并认为其以持续或反复发作的严重疲劳为主要特征,多伴有关节酸困、睡眠紊乱及抑郁等多种躯体及精神神经症状<sup>[1,2]</sup>。对此国内进行了大规模的临床流行病学调研,发现 70%以上人群处于亚健康状态,且其临床表现复杂多样,但疲劳症状的出现频率高居榜

首<sup>[3,4]</sup>。因此探寻其可靠的防治措施成为必要。保元煎剂为第四军医大学西京医院中医科李锋教授等多年临证亚健康人群证实行之有效的结晶,在前期临床观察中发现保元煎剂对亚健康人群有良好的防治作用<sup>[5]</sup>。为深入研究保元煎剂防治亚健康人群的作用机制,本实验拟从保元煎剂对疲劳大鼠力竭游泳时间以及 ATP、羟自由基、乳酸含量的影响进行观察,现汇报如下。

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## 1 材料和方法

### 1.1 动物与药物

健康雄性清洁级 SD 大鼠 60 只,体重 150 g-180 g,购自第四军医大学实验动物中心。保元煎剂由第四军医大学药物研究所提供。

### 1.2 试剂与仪器

塑料游泳池(100 cm× 60 cm× 80 cm);电子称;砝码;37℃ 恒温水浴锅;计时器;722 型分光光度计;BECKMAN 超速离心机;ATP 含量测试盒、羟自由基测定试剂盒、乳酸测试盒购自南京建成生物有限公司。

### 1.3 动物分组与造模

实验动物适应性喂养 3 d 后,于 20:00 负 5%体质量砝码适应性游泳 10 min,连续 3 d(水深 50 cm,水温 24± 2℃),后随机分为 6 组:空白组、模型组、保元煎剂低、中、高剂量组,西洋参组。参照文献方法<sup>[6]</sup>采用力竭游泳复合睡眠剥夺构建亚健康疲劳大鼠模型。除正常组外,于每日 20:00 负 5%体质量砝码行力竭游泳,并记录力竭游泳时间(以鼻尖没入水下 10 s 不能自行浮起为度)<sup>[7]</sup>,后放入水深 1.5 cm 大鼠饲养笼(大鼠不能逃脱且可自由进食饮水)20 h,20 h 后大鼠常规饲养,连续 4 d。

### 1.4 给药

分组后,各组大鼠于每日行力竭游泳前 1 h 给药,保元煎剂低、中、高剂量组给药量分别为 1.04 g·kg<sup>-1</sup>·d<sup>-1</sup>、2.08 g·kg<sup>-1</sup>·d<sup>-1</sup>、4.15 g·kg<sup>-1</sup>·d<sup>-1</sup>,西洋参组给药量为 0.27 g·kg<sup>-1</sup>·d<sup>-1</sup>,正常组给予同体积蒸馏水作为安慰对照,每天 1 次,连续给药 4 d。

### 1.5 ATP、乳酸、自由基指标检测

大鼠末次睡眠剥夺后,腹腔注射水合氯醛(300 mg·kg<sup>-1</sup>)麻醉,眼球采血,按试剂盒说明测定。

### 1.6 统计学处理

实验结果以均数± 标准差( $\bar{x} \pm s$ )表示,采用 SPSS13.0 统计软件进行统计分析,组间差异采用单因素方差分析, P<0.05 为差异显著,有统计学意义。

## 2 结果

### 2.1 力竭游泳时间测定结果

与模型组比较,西洋参组、保元煎剂低、中、高剂量组力竭游泳时间显著延长(P<0.05),与西洋参组比较,保元煎剂高剂量组力竭游泳时间显著延长(P<0.05)。见表 1。

表 1 保元煎剂对疲劳大鼠力竭游泳时间的影响( $\bar{x} \pm s$ )

Table 1 The effects of BaoYuan decoction on exhausted swimming time of fatigue rats( $\bar{x} \pm s$ )

| Group                         | Exhausted swimming time(min) |
|-------------------------------|------------------------------|
| Normal group                  | 29.71± 2.31★                 |
| Model group                   | 15.77± 1.08                  |
| American ginseng group        | 20.94± 0.94★                 |
| Low-dose BaoYuan decoction    | 16.07± 0.65★                 |
| Middle-dose BaoYuan decoction | 23.21± 1.16★                 |
| High-dose BaoYuan decoction   | 27.34± 1.46★△                |

Note: Compared with model group, ★ P<0.05; Compared with American ginseng group, △ P<0.05.

### 2.2 ATP、乳酸、自由基含量测定结果

与模型组比较,西洋参组、保元煎剂低、中、高剂量组 ATP 含量明显升高,自由基减少(P<0.05);保元煎剂中、高剂量组乳酸含量降低(P<0.05);与西洋参组比较,保元煎剂低、中、高剂量组 ATP 含量升高(P<0.05),保元煎剂中剂量组自由基含量下降(P<0.05),保元煎剂高剂量组乳酸含量下降(P<0.05)。见表 2。

表 2 保元煎剂对疲劳大鼠自由基、乳酸、ATP 的影响( $\bar{x} \pm s$ )

Table 2 The effects of BaoYuan decoction on ATP, Hydroxyl radical and Lactic acid of fatigue rats( $\bar{x} \pm s$ )

| Group                         | Hydroxyl radical (IU/mL) | Lactic acid (μmol/L) | ATP(μmol/L)   |
|-------------------------------|--------------------------|----------------------|---------------|
| Normal group                  | 64.11± 1.73★             | 1263.10± 105.71★     | 25.50± 1.02★  |
| Model group                   | 88.95± 1.58              | 1980.19± 284.10      | 17.13± 1.60   |
| American ginseng group        | 69.82± 1.21★             | 1717.47± 187.03      | 23.94± 0.77★  |
| Low-dose BaoYuan decoction    | 68.08± 0.74★             | 1530.63± 138.52      | 26.93± 0.71★△ |
| Middle-dose BaoYuan decoction | 64.54± 1.43★△            | 1266.56± 158.32★     | 28.11± 1.49★△ |
| High-dose BaoYuan decoction   | 57.17± 0.78★△            | 1026.10± 111.15★△    | 34.79± 2.01★△ |

Note: Compared with model group, ★ P<0.05; Compared with American ginseng group, △ P<0.05.

## 3 讨论

祖国传统中医学并无“亚健康”一词,但在《黄帝内经》中论述有“未病”——“上工不治已病,治未病”,两者范畴相近<sup>[8]</sup>。我院中医科多位专家教授根据中医传统理论,结合多年临床经验,认为亚健康的根本病机在于肝郁肾虚。祖国传统医学认为肝主藏血,为“罢极之本”、主筋,“诸筋者皆属于节”,多实;肾藏精,为“先天之本”、主骨生髓,多虚;二脏“荣则同荣、衰则同衰”。亚健康持续或反复发作的严重疲劳,以及伴随的记忆力减退、关

节酸困、睡眠紊乱、抑郁等躯体及精神症状,与中医肝郁、肾虚所出现的临床症候群相类似。“保元煎”正是以上述理论为基础,以“舒肝保元”为基本治则组方,从而使其与亚健康之根本病机“肝郁肾虚”相吻合,与中医“治未病”思想相吻合。保元煎剂以入心、肺、肾经的西洋参为君药,从而大补元气,固肾保精,益精养血<sup>[9]</sup>。西洋参可用于阴虚火旺、气阴两伤,无力、烦倦、口渴、健忘等证。以黄芪,五味子,麦冬,山药为臣,一取黄芪补气升阳,益卫固表之功,与君药西洋参相须为用补内外之气,缓解易疲劳、多汗之证;二取麦冬山药相使为用养阴生津,助君药西

洋参益气生津,治疗口渴舌红、虚烦;三取五味子敛肺滋肾,生津敛汗,涩精止泻、宁心安神之功。因肾为气之根,故取五味子益肾固元敛肺。助君药西洋参,臣药麦冬、山药生津敛汗并能宁心安神。以酸枣仁为佐,取酸枣仁安神之功助五味子宁心安神。以柴胡、桔梗为使,疏肝解郁,宣通内外、条达上下,疏利三焦、扶正祛邪、调和诸药,使诸药补而不滞。诸药合用,有“益气保元、舒肝安神”之功。

中药药理学研究亦可在一定程度上对保元煎剂调治亚健康的作用机制做出一些解释:如西洋参皂苷具有很好的中枢神经系统抗疲劳、镇静及抗缺氧作用,西洋参根多糖还具有提高机体免疫力、扶正固本的作用<sup>[10]</sup>;麦门冬多糖可促进细胞免疫和体液免疫,从而具有良好的免疫增强和刺激作用,此外麦冬煎剂、麦冬水提取物、麦冬注射液尚有改善小鼠耐缺氧能力的作用<sup>[11]</sup>;五味子可提高能量代谢中的锰超氧化物歧化酶、NADH 脱氢酶以及 ATP 酶活性,降低 MDA 含量,使衰老小鼠脑线粒体能量代谢改变延缓,从而保护脑线粒体,五味子多糖亦有良好的保肝作用<sup>[12]</sup>;山药可延长免疫机能低下小鼠的缺氧耐受时间,降低血清中血糖与血脂含量,升高肝糖元和心肌糖元含量,从而有利于提高对血糖的利用<sup>[13]</sup>;酸枣仁总皂苷及黄酮类成分均有镇静催眠及提高细胞耐缺氧能力的作用<sup>[14]</sup>,且酸枣仁与五味子同用可起到协同作用<sup>[15]</sup>;柴胡皂甙有良好的保肝作用,并能通过抑制大脑组织海马区胆碱乙酰转移酶蛋白表达、降低大脑海马区乙酰胆碱酯酶活性和减少大脑海马区神经细胞凋亡而引起抗抑郁作用<sup>[16]</sup>;桔梗有一定的增强免疫力及保肝作用<sup>[17]</sup>。

本实验研究结果揭示出了相对于正常组,保元煎剂可明显提高疲劳大鼠 ATP 水平,同时相较于西洋参组,可降低疲劳物质乳酸、羟自由基蓄积。ATP 是生物体内能量的直接利用形式,ATP 含量的高低可直接衡量机体内能量充足与否<sup>[18]</sup>,提示保元煎剂可升高疲劳大鼠 ATP 水平从而保证能量供应。乳酸既是糖酵解的产物,又是有氧代谢氧化的底物,还可经糖异生途径转变为糖。当血乳酸过高时,可使血液 pH 值下降,使得与机体能量代谢有关的酶活性下降,导致疲劳的发生<sup>[19]</sup>。羟自由基性质十分活跃,可作用于细胞膜发生脂质过氧化反应,使膜内外离子交换障碍,导致细胞寿命缩短或死亡<sup>[20]</sup>。保元煎剂可通过降低血乳酸、羟自由基含量,保证细胞的正常形态及正常的能量代谢,从而降低疲劳的发生。从上我们可看出保元煎剂在能量物质代谢正、反两方面均起到了很好的抗疲劳作用。这在一定程度上说明了“疏肝保元”治则对于缓解亚健康疲劳状态的合理性,同时我们亦可看出以中医理论为指导的中药复方相较于中药单体作用的多向性,疗效的确切性,从而为我们继承和发展中医药这座宝库提供了有力的依据。

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