

doi: 10.13241/j.cnki.pmb.2021.06.006

补肾填精法对脑卒中失眠症模型大鼠血清褪黑素含量表达的影响*

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摘要 目的:探讨补肾填精法对脑卒中失眠症模型大鼠血清褪黑素(Melatonin, MT)含量表达的影响。**方法:**24只建模成功的脑卒中失眠症大鼠随机平分为3组-模型组、低剂量组、高剂量组。于造模第7d起,三组分别给予生理盐水及补肾填精汤(低剂量组最终含生药浓度低剂量为0.7 g/mL、中剂量为1.4 g/mL)以1 mL/(100 g·d)灌胃,连续14 d,记录血清MT表达变化情况。**结果:**三组治疗第1 d、第7 d与第14 d的逃避潜伏期在组内与组间对比差异无统计学意义($P>0.05$)。低剂量组、高剂量组治疗第7 d与第14 d的进入中央区次数多于模型组($P<0.05$),也多于治疗第1 d($P<0.05$),高剂量组也多于低剂量组($P<0.05$)。低剂量组、高剂量组治疗第14 d的血清MT含量、去甲肾上腺素转运蛋白与褪黑素受体蛋白相对表达水平高于模型组($P<0.05$),高剂量组高于低剂量组($P<0.05$)。**结论:**补肾填精法在脑卒中失眠症模型大鼠中的应用能促进血清MT的释放,提高去甲肾上腺素转运蛋白与褪黑素受体蛋白的表达,从而促进缓解失眠症状。

关键词:补肾填精;脑卒中;失眠症;褪黑素;去甲肾上腺素转运蛋白

中图分类号:R-33;R743;R242 **文献标识码:**A **文章编号:**1673-6273(2021)06-1028-04

Effect of Tonifying Kidney and Replenishing Essence Method on Expression of Serum Melatonin in Stroke and Insomnia Model Rats*

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ABSTRACT Objective: To investigate the effect of the method of invigorating the kidney and replenishing essence on the expression of serum melatonin (MT) in rat model of stroke and insomnia. **Methods:** A total of 24 rats of successfully modeled stroke and insomnia were randomly and equally divided into three groups-model group, low-dose group, and high-dose group. On the 7th day of model establishment, the three groups were given physiological saline and Tonifying Kidney and Replenishing Essence Decoction (the final low-dose group containing crude drug concentration was 0.7 g/mL, the medium dose was 1.4 g/mL) at a rate of 1 mL/ (100 g·d) Gavage for 14 consecutive days. The changes in serum MT expression were recorded. **Results:** There was no significant difference in the escape latency among the three groups on the 1st, 7th and 14th day of treatment ($P>0.05$). The low-dose group and high-dose group had more access to the central area on the 7th and 14th day of treatment than the model group ($P<0.05$), and more than the 1st day of treatment ($P<0.05$), and the high-dose group was also more than the low-dose group ($P<0.05$). The serum MT content, the relative expression levels of norepinephrine transporter and melatonin receptor protein on the 14th day of treatment in the low-dose and high-dose groups were higher than those in the model group ($P<0.05$), and the high-dose group was higher than the low-dose group ($P<0.05$). **Conclusions:** The application of the method of Tonifying Kidney and Replenishing Essence in stroke and insomnia model rats can promote the release of serum MT, increase the expression of norepinephrine transporter and melatonin receptor protein, thereby alleviating insomnia symptoms.

Key words: Tonifying Kidney and Replenishing Essence; Stroke; Insomnia; Melatonin; Norepinephrine transporter

Chinese Library Classification (CLC): R-33; R743; R242 **Document code:** A

Article ID: 1673-6273(2021)06-1028-04

前言

失眠症是一种不能获得正常睡眠为特征的病症,可导致患

者出现体倦乏力、反应迟钝等症状,严重影响患者的身心健康^[1,2]。现代医学治疗失眠症多采用苯二氮卓类药物,具有起效快速、服用便利的优势,但是长期服用也容易产生戒断反应、耐

* 基金项目:林培政广东省名中医传承工作室项目(211020030624)

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(收稿日期:2020-09-22 接受日期:2020-10-18)

受性、成瘾性等不足,也会出现一定的不良反应^[3]。同时部分患者容易拒绝服西药而耽误病情,导致治疗效果不佳^[45]。失眠症的发病机理还不明确,不过与脑卒中的关系密切,脑卒中可导致机体发生氧化与抗氧化失衡,从而引起细胞、组织的氧化损伤,可诱发失眠症的发生^[67]。中医在治疗失眠上有很大的优势,其注重辩证与整体治疗^[8]。补肾填精汤剂由地黄、龟板、龙骨、牡蛎、白芍、当归、郁金、酸枣仁等组成,功在疏肝解郁、安神定志^[9]。褪黑素(Melatonin, MT)为一种典型的抑制性激素,具有镇静安神作用。MT分泌异常时,可令人兴奋,导致睡卧不安、入睡困难^[10-12]。本文具体探讨了补肾填精法对脑卒中失眠症模型大鼠血清MT含量表达的影响,以明确中药治疗的价值与效果。现总结报道如下。

1 资料与方法

1.1 研究对象

SPF级成年雄性 Sprague Dawley(SD)大鼠(30只)购自成都达硕动物科技有限公司提供(动物合格证编号 00023343, 4~5周龄,体重 140~160 g,饲养环境:温度 22℃~24℃, 40 cm×22 cm×20 cm 大小的动物饲养笼,相对湿度 40%~60%,饮食与饮水自由, 12/12 h 光照黑暗循环)。

MT 酶联免疫检测试剂盒(Sigma 公司,美国), 十字迷宫视频跟踪系统(成都泰盟科技有限公司), 对氯苯丙氨酸(宝生物工程有限公司), 抗去甲肾上腺素转运蛋白抗体与抗褪黑素受体蛋白抗体(Sigma 公司,美国)。

1.2 动物建模与分组

所有大鼠都采用了改良双侧颈总动脉分次结扎的方法制备大鼠脑卒中模型,然后采用慢性夹尾刺激和氯苯丙氨酸腹腔注射复合因子造模法检测脑卒中失眠症模型大鼠。用尖端包扎纱布的止血钳分别夹大鼠尾部,将每组大鼠置于同一笼内,令其与其他大鼠厮打(以不破皮流血为度),刺激 30 min 左右,连续 20 d。

造模成功标准:大鼠跨越平台时间(逃避潜伏期)显著延长精神变差,反应迟钝,高架十字迷宫实验进入中央区次数少于≤8次。

30只大鼠中有24只建模成功,随后随机平分为3组-模型组、低剂量组、高剂量组。于造模第7d起,三组分别给予生理盐水及补肾填精汤(低剂量组最终含生药浓度低剂量为0.7 g/mL,中剂量为1.4 g/mL)以1 mL/(100 g·d)灌胃,连续14 d。补肾填精汤组成:珍珠母 30 g、生地黄 15 g、龟板 10 g、生龙骨 15 g、生牡蛎 15 g、白芍 15 g、当归 15 g、郁金 15 g、酸枣仁 15 g、龟板 10 g,大鼠用量为成人的6倍,按传统煎药方法煎煮,浓缩灭菌。

1.3 观察指标

(1)在治疗第1 d、第7 d与第14 d进行水迷宫实验,记录逃避潜伏期。在水迷宫装置自动记录大鼠从入水到寻台成功的时间,即逃避潜伏期,如120 s大鼠仍未找到平台,则潜伏期为120 s。(2)在上述同一时间点进行高架十字迷宫实验,记录大鼠进入中央区次数。(3)在治疗第14 d处死大鼠,用抗凝管采集大鼠股动脉血液标本,分离血清,采取酶联免疫法检测血清MT含量。(4)取处死大鼠的海马组织,提取总蛋白,采用 Western blot 法检测去甲肾上腺素转运蛋白与褪黑素受体蛋白相对表达水平,以 β -actin 作为内标。

1.4 统计学方法

选择 SPSS 19.00 软件进行数据处理,计量数据选择均数±标准差进行表示,计数数据采用百分比进行表示,本研究多组间数据对比用 one-way ANOVA 分析,两两比较用 t-test 分析与卡方分析等,检验水准为 $\alpha=0.05$ 。

2 结果

2.1 逃避潜伏期对比

三组治疗第1 d、第7 d与第14 d的逃避潜伏期在组内与组间对比差异无统计学意义($P>0.05$),见表1。

表1 三组治疗不同时间点的逃避潜伏期对比($s, \bar{x} \pm s$)

Table 1 Comparison of escape latency among three groups at different time points of treatment ($s, \bar{x} \pm s$)

Groups	n	1 d	7 d	14 d
Model group	8	78.22± 12.48	77.22± 14.29	77.29± 11.42
Low-dose group	8	78.11± 13.72	78.77± 14.33	78.76± 13.77
High dose group	8	78.98± 11.47	78.67± 12.10	80.76± 12.11
F		0.482	0.569	0.774
P		0.655	0.514	0.376

2.2 高架十字迷宫实验结果对比

低剂量组、高剂量组治疗第7 d与第14 d的进入中央区次数多于模型组($P<0.05$),也多于治疗第1 d($P<0.05$),高剂量组也多于低剂量组($P<0.05$),见表2。

2.3 血清 MT 含量对比

低剂量组、高剂量组治疗第14 d的血清MT含量高于模型组($P<0.05$),高剂量组高于低剂量组($P<0.05$),见表3。

2.4 去甲肾上腺素转运蛋白与褪黑素受体蛋白相对表达水平

对比

低剂量组、高剂量组治疗第14 d海马组织的去甲肾上腺素转运蛋白与褪黑素受体蛋白相对表达水平高于模型组($P<0.05$),高剂量组高于低剂量组($P<0.05$),见表4。

3 讨论

脑卒中是中老年人群的常见病、多发病,可给患者家庭和社会带来越来越沉重的负担^[13]。脑卒中患者多伴随有失眠症,

表 2 三组治疗不同时间点的进入中央区次数对比(次, $\bar{x} \pm s$)

Table 2 Comparison of number of entering central area among three groups at different time points of treatment (times, $\bar{x} \pm s$)

Groups	n	1 d	7 d	14 d
Model group	8	5.78 $\pm$ 0.11	5.81 $\pm$ 0.08	5.78 $\pm$ 0.14
Low-dose group	8	5.82 $\pm$ 0.13	11.73 $\pm$ 1.44 [#]	13.77 $\pm$ 1.75 [#]
High dose group	8	5.80 $\pm$ 0.18	13.09 $\pm$ 1.11 ^{*#}	15.98 $\pm$ 0.84 ^{*#}
F		0.123	9.113	12.049
P		0.899	0.001	0.000

Note: Compared with the model group, [#] $P < 0.05$; compared with the high-dose group, ^{*} $P < 0.05$.

表 3 三组治疗第 14 d 的血清 MT 含量对比(pg/mL, $\bar{x} \pm s$)

Table 3 Comparison of serum MT content among three groups on 14 th day of treatment (pg/mL, $\bar{x} \pm s$)

Group	n	MT
Model group	8	36.22 $\pm$ 2.19
Low-dose group	8	44.98 $\pm$ 1.48 [#]
High dose group	8	54.87 $\pm$ 1.73 ^{*#}
F		12.044
P		0.000

Note: Compared with the model group, [#] $P < 0.05$; compared with the high-dose group, ^{*} $P < 0.05$.

表 4 三组治疗第 14 d 的海马组织去甲肾上腺素转运蛋白与褪黑素受体蛋白相对表达水平对比($\bar{x} \pm s$)

Table 4 Comparison of relative expression levels of norepinephrine transporter and melatonin receptor protein in hippocampus on 14th day of treatment among three groups ($\bar{x} \pm s$)

Groups	n	Norepinephrine transporter	Melatonin receptor protein
Model group	8	0.87 $\pm$ 0.03	1.37 $\pm$ 0.08
Low-dose group	8	2.01 $\pm$ 0.04 [#]	3.82 $\pm$ 0.06 [#]
High dose group	8	5.62 $\pm$ 0.13 ^{*#}	8.24 $\pm$ 0.22 ^{*#}
F		15.002	17.584
P		0.000	0.000

Note: Compared with the model group, [#] $P < 0.05$; compared with the high-dose group, ^{*} $P < 0.05$.

二者存在高度共病性,可相互先后发生,属于双向关系^[14]。药物治疗为失眠症患者的主要治疗方法,主要是选用具有促眠、镇静类作用的人工合成西药,虽然有一定的疗效,但是可降低患者的社会功能与生活质量^[15,16]。补肾填精法是临证经验总结的针对失眠症的有效方剂,其功用安神定志、疏肝解郁^[17],该方重用地黄、龟板、龙骨、牡蛎、白芍、当归、郁金、酸枣仁等组方,具有抗焦虑、抗抑郁等作用,也可养心安神以缓解气郁化火^[18]。失眠是应激性刺激后难以避免的一种常见情绪反应,可表现为探究性行为的减少,伴有惊吓、呆滞等,而高架十字迷宫实验是评判大鼠失眠状态的重要检测方法,特别是中央区进入次数可以良好反映大鼠是否存在失眠状态^[19]。本研究显示三组治疗第 1 d、第 7 d 与第 14 d 的逃避潜伏期在组内与组间对比差异无统计学意义;低剂量组、高剂量组治疗第 7 d 与第 14 d 的进入中央区次数多于模型组,也多于治疗第 1 d,高剂量组也多于低剂量组,表明补肾填精法在脑卒中失眠症模型大鼠中的应用能改善失眠症状。以往的研究中,补肾填精汤主要用于治疗慢性前列腺炎合并勃起功能障碍^[20]、卵巢功能低下^[21]的研究中,能有效促

进患者临床症状缓解,减轻焦虑,抑郁等负性心理,提高生活质量,从而改善患者的睡眠状态。

任何导致阳不入阴的原因均可引起失眠,寻找导致患者失眠的根本原因并掌握基本病机,辩证论治,能取得比较好的治疗效果^[22]。补肾填精之品专攻补肾,可起到重镇安神、养心安神的效果^[23]。褪黑素是在哺乳动物及人体松果体中分泌的主要激素,在预防皮层功能紊乱、调节人体免疫功能等过程中发挥重要作用^[24,25]。褪黑素可通过抑制一氧化氮合酶的表达,从而发挥抗自由基和抗过氧化损伤作用,促进缓解失眠症状^[26,27]。本研究显示低剂量组、高剂量组治疗第 14 d 的血清 MT 含量高于模型组,高剂量组高于低剂量组,表明补肾填精法在脑卒中失眠症模型大鼠中的应用能促进血清 MT 的释放。

海马组织是控制昼夜节律的关键部位,也是研究睡眠的重要观察点^[28,29]。并且海马是人体记忆功能的活动中枢,对于脑组织的缺氧、缺血等损伤极为敏感^[30,31]。本研究显示低剂量组、高剂量组治疗第 14 d 海马组织的去甲肾上腺素转运蛋白与褪黑素受体蛋白相对表达水平高于模型组,高剂量组高于低剂量

组,表明补肾填精法在脑卒中失眠症模型大鼠中的应用能提高去甲肾上腺素转运蛋白与褪黑素受体蛋白的表达。本研究的结果可以为脑卒中失眠症患者的治疗提供新思路和方法,后续可以在临床中进行试验探究。

总之,我们的研究表明补肾填精法在脑卒中失眠症模型大鼠中的应用能促进血清 MT 的释放,提高去甲肾上腺素转运蛋白与褪黑素受体蛋白的表达,从而有助于促进缓解失眠症状。本研究也有一定的不足,因补肾填精汤是混合物,其药理机制分析还比较困难,且本研究没有设置空白对照,可能存在研究偏倚,将在后续研究中深入分析。

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