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七氟醚对脑缺血再灌注损伤大鼠认知功能及海马 S100β 及 PGRN 表达的影响 *

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摘要 目的:探索用七氟醚预处理后脑缺血再灌注损伤大鼠的认知功能变化情况以及海马细胞内钙结合蛋白（S100β）及颗粒蛋白前体（Progranulin,,PGRN）的表达水平。**方法:**选取 SPF 级雄性大鼠 36 只,按照随机数字表法分为假手术组（A 组）、脑缺血再灌注损伤组（B 组）、七氟醚预处理组（C 组）,每组 12 只。B 组和 C 组大鼠采用线栓法建立脑缺血再灌注损伤模型,缺血 2 h,再灌注 24 h,假手术组仅切开不插入线栓。术前七氟醚预处理组大鼠吸入体积分数 3 %七氟醚和氧流量为 2 L/min 的混合气体,持续 1 h,假手术组和脑缺血再灌注损伤组单纯吸入 2 L /min 的氧气。建模完成后采用大鼠神经功能缺损评分（modified neurological severity score,mNSS）评估大鼠的神经功能状况;Morris 水迷宫实验检测各组大鼠学习认知功能;Western Blot 检测各组大鼠 S100β 和 PGRN 的表达情况。**结果:**（1）B、C 组大鼠 mNSS 评分显著高于 A 组,C 组评分较 B 组有明显降低（$P<0.05$）;（2）与 A 组相比 B、C 两组逃逸潜伏期明显延长,C 组与 B 组相比逃逸潜伏期显著缩短（$P<0.05$）;（3）B、C 组大鼠海马 S100β 和 PGRN 的表达较 A 组有显著上调,其中 C 组 S100β 表达较 B 组显著降低,PGRN 表达显著升高（$P<0.05$）。**结论:**本文通过观察七氟醚预处理对 CIRI 大鼠认知功能及海马 S100β 和 PGRN 蛋白表达水平的影响,发现七氟醚预处理对 CIRI 大鼠认知功能有显著提高,其作用机制与海马 S100β 和 PGRN 的表达密切相关,为进一步探索其作用机制提供参考证据。

关键词:脑缺血再灌注损伤;七氟醚;认知功能;S100β;PGRN

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Effects of Sevoflurane on Cognitive Function in Rats with Cerebral Ischemia Reperfusion Injury and the Expression of the S100β and PGRN in Hippocampus*

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ABSTRACT Objective: To investigate the changes of cognitive function and the expression levels of calcium-binding protein (S100β) and granule-protein precursor (PGRN) in hippocampal cells of rats pretreated with sevoflurane after cerebral ischemia reperfusion injury. **Methods:** 36 SPF male rats were selected and divided into sham operation group (group A), cerebral ischemia reperfusion injury group (group B) and sevoflurane pretreatment group (group C) according to random number table method, with 12 rats in each group,Cerebral ischemia/reperfusion injury models were established in group B and group C by wire embolization method. The rats were treated with ischemia for 2h and reperfusion for 24h. The sham operation group was only cut without inserting wire embolization. Rats in the sevoflurane preconditioning group were inhaled a mixture of sevoflurane with a volume fraction of 3% and an oxygen flow of 2 L /min for 1h, while the sham operation group and the cerebral ischemia/reperfusion injury group were simply inhaled oxygen of 2 L/min. After the modeling, the neurological function of the rats was evaluated by the Rat Neurological Deficiency Score (MNSS). Morris water maze test was used to detect the learning and cognitive function of rats in each group;The expression of S100βand pGRN in each group was detected by Western Blot. **Results:** (1)Mnss score in groups B and C was significantly higher than that in group A, and score in group C was significantly lower than that in group B ($P<0.05$).(2)Compared with group A, the escape latency of group B and group C was significantly longer, and the escape latency of group C was significantly shorter than that of group B ($P<0.05$). (3)Compared with group A, the expression of S100β and pGRN in the hippocampus of rats in groups B and C were significantly up-regulated, and the expression of S100βin group C was significantly decreased compared with group B, while the expression of pGRN was significantly in-

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creased (P<0.05). **Conclusion:** In this study, the effects of sevoflurane pretreatment on cognitive function and the protein expression levels of S100β and PGRN in hippocampus of CIRI rats were observed. The results show that sevoflurane pretreatment significantly improved the cognitive function of CIRI rats, and the mechanism of action was closely related to the expression of S100β and PGRN in hippocampus, which provided evidence for further exploration of the mechanism of action.

Key words: Cerebral ischemia reperfusion injury; Sevoflurane; Cognitive function; S100β; PGRN

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前言

脑缺血再灌注损伤（cerebral ischemiareperfusion injury, CIRI)是临床治疗缺血性脑卒中溶解血栓、恢复血液灌注后,时常出现的缺血性损伤进一步加重的现象,更有甚者脑组织在缺血一定时间恢复血流后发生不可逆性损伤,严重威胁人类生命健康[1]。脑缺血再灌注损伤对海马组织的影响更为明显[2],可加速海马区细胞的凋亡、导致学习和记忆能力等认知功能的损害[3,4],严重影响患者的生活质量。七氟醚是一种临床应用较广的吸入麻醉剂,研究表明[5,6]经七氟醚预处理可以明显减轻脑缺血再灌注损伤,其作用机制还需探索。S-100β 蛋白是细胞内钙结合蛋白 S100 家族最具活性的成员之一，相对分子量为 21× 10^3,主要存在于中枢神经系统中[7],难以通过血脑屏障,表现出明显的浓度特异性。大量研究证明[8-10]血清 S-100β 蛋白浓度与脑损伤程度呈明显相关性,损伤程度加深时,S-100β 蛋白通过受损血脑屏障进人外周血,是目前反映脑损伤程度的关键生化标志物。颗粒蛋白前体(progranulin,PGRN)是一种由 Grn 基因编码的具有多种生理功能的分泌性糖蛋白,参与机体生长发育、细胞增殖、损伤修复以及炎性反应等过程[11]。研究发现[12-14]其通过抑制炎症反应、减轻血脑屏障的破坏保护脑缺血损伤大鼠的生理功能,促进脑内神经再生对大鼠认知功能与学习记忆能力有显著改善作用。因此,本研究通过探索经七氟醚预处理后 CIRI 大鼠学习记忆能力改变，以及海马区 S-100β、PGRN 蛋白的表达情况,为作用机制研究提供新的思路。

1 资料与方法

1.1 试剂与仪器

试剂:七氟醚(上海将来实业股份有限公司,规格 500 mL,批号 81001)，线栓（美国 Doccol，型号 403756PK5Re),Anti-S100beta 抗体(上海谷研实业有限公司,规格 0.2 mL/200 mg),Anti-progranulin/PGRN 抗体(深圳子科生物科技有限公司,规格:0.1 mL/0.2 mL),蛋白测定试剂盒、提取试剂盒北京百奥莱博科技有限公司，凝胶配制试剂盒上海万生昊天生物技术有限公司,通用型抗体稀释液购自上海雅酶生物科技有限公司。

仪器：动物麻醉机（山东博科科学仪器有限公司，型号 ZS-MX-HX)，麻醉气体监测仪（北京康高特仪器设备有限公司,日本 RIKEN 理研,型号 FI-21),电泳仪(北京六一生物科技有限公司,型号 DYCP-32B),多功能酶标仪(美国赛默飞世尔,型号 Multiskan FC)。

1.2 实验方法

1.2.1 **试验动物** 选取健康雄性 SD 大鼠 36 只,均为 SPF 级,体重 230~280 g,平均(258.6± 7.5)g,所有动物购自中国科学院上海实验动物中心，动物合格证编号:SLAC.SD No00050023。保持室温 22～25℃、相对湿度 47 %～65 %。按照随机数字表法将所有动物分成 3 组,每组 12 只,假手术组(A 组),脑缺血再灌注组(B 组),七氟醚预处理组(C 组)。

1.2.2 **模型建立** 试验前所有动物进行 3 d 适应性喂养后,模型组和七氟醚预处理组采用线栓法[10]建立大鼠脑缺血再灌注损伤模型。首先用 10 %水合氯醛 0.30 g/kg 腹腔注射进行麻醉,将大鼠呈仰卧姿势固定在手术台上,颈旁正中切开分离右侧颈总动脉和颈内动脉,结扎颈内动脉阻断血流,在颈外动脉做个小切口,插入线栓,沿颈内动脉推动至有阻力为止,缝合伤口并进行防感染处理。缺血 2 h 之后,拔出线栓再灌注 24 h。术中保持大鼠呼吸通畅、体温恒定(肛温 37℃左右)。假手术组大鼠只切开不结扎也不插线栓。本实验中大鼠双侧颈总动脉血管被剪断,即造模成功。

1.2.3 **给药方式** 七氟醚组预处理,将 C 组大鼠置于铺有钠石灰的密闭麻醉箱中,适当位置放置取暖器,维持大鼠肛温 37 ℃左右。进气口吸入七氟醚及氧气混合气体,3 %的七氟醚、氧流量 2 L/min,持续 1 h。麻醉过程监测大鼠呼吸状况及出气口麻醉剂、其他气体浓度,麻醉结束后将大鼠置于加热垫上自然清醒,并放回鼠笼中。A 组和 B 组单纯吸入 2 L/min 氧气,保持体温稳定。

1.3 检测方法

神经功能缺损评分[15]造模完成后大鼠参考 mNSS 评价大鼠的运动、平衡、反射等神经功能,根据大鼠具体表现症状进行评分,如:提尾悬空时不能完全伸展对侧前肢,行走时对侧倾倒、转圈,甚至不能行走、意识模糊等,分值范围 0～18 分,症状越严重评分越高,无神经功能缺损表现评分为 0 分。

Morris 水迷宫实验[16-18]:实验周期 7 d,训练阶段连续进行 6 d,每天训练 8 次,于第 7 d 测定记录大鼠寻找平台所耗时间。具体操作方法:选取长 120 cm,高 50 cm,深 30 cm 的水槽,分成 4 个部分,训练时将 2 cm 的平台轮流放置在 4 部分,训练大鼠寻找池底的平台，逃逸潜伏期以 60 s 为准,60 s 内找到平台记录实际时间,超过 60 s 则记 60 s 为逃逸潜伏期。撤除平台后测定后,大鼠从离平台最远位置出发 60 s 内穿越平台次数。

Western Blot 制备：各组大鼠在深度麻醉情况下进行断头取脑,在低温条件下快速剥离海马组织,精确称重后制成 10 % 的组织匀浆,每 100 mg 组织中加入 1.5 mL 提前配制好的全蛋白提取液（每 1 mL 冷蛋白裂解液中加入 10 μL 浓度为 100 mmoL/L 的 PMSF、1 μL蛋白酶抑制剂以及 10 μL磷酸酶抑制剂),研磨 1 min 后离心,吸取上清液,采用 BCA 法测定蛋白浓度。配制聚丙烯酰胺凝胶(10 %分离胶,4 %积层胶) ,灌胶时避免气泡产生,上样后电泳 100 V 30 min、120 V 50 min,切取目

标凝胶区域转至 PVDF 膜 100 V 2 h，5 % 封闭液封闭 2 h，加入兔抗鼠 S-100β 抗体(1: 2000)，兔抗鼠 PGRN 抗体(1: 2000)，用 TBST 稀释后与膜在 4℃过夜，TBST 清洗 3 次，5 min / 次，加 HRP 标记的山羊抗兔 IgG（1∶5000）清洗方法同上，室温孵育 1 h，滴加 ECL 发光液显影成像，测量各膜的灰度值。

1.4 统计学方法

采用 SPSS 20.0 软件对试验数据进行分析，计量指标以均数± 标准差($\bar{x}\pm s$)表示，采用单因素方差分析进行组间比较，$P<0.05$ 时，说明组间差异有统计学意义。计数资料以百分率 n%表示，组间比较用 x^2 检验。

2 结果

2.1 神经功能缺损评分

比较各组大鼠神经功能缺损 mNSS 评分结果发现，脑缺血再灌注损伤组和七氟醚组大鼠 mNSS 评分显著高于假手术组，且七氟醚预处理组大鼠评分较脑缺血再灌注组有明显降低（$P<0.05$），见表 1。

表 1 大鼠神经功能缺损 mNSS 评分比较($\bar{x}\pm s$)

Table 1 Comparison of MNSS scores of nerve function deficit in rats($\bar{x}\pm s$)

Groups	mNSS scores
Sham operation group（A,n=12）	0.62± 0.03
CIRI group（B,n=12）	14.78± 2.15*
Sevoflurane group（C,n=12）	6.64± 1.72*&

Note: Compared with Sham group, *$P<0.05$; compared with CIRI group, &$P<0.05$.

2.2 Morris 水迷宫实验

Morris 水迷宫实验结果表明，脑缺血再灌注损伤组和七氟醚组大鼠与假手术组相比逃逸潜伏期明显延长，且与脑缺血灌注组相比七氟醚组逃逸潜伏期显著缩短（$P<0.05$），见表 2。

表 2 大鼠逃避潜伏期和穿越平台次数比较($\bar{x}\pm s$)

Table 2 Comparison of the escape incubation period and number of crossing platforms($\bar{x}\pm s$)

Groups	Escape incubation period	Number of crossing platforms
Sham operation group（A,n=12）	36.62± 7.63	4.35± 0.56
CIRI group（B,n=12）	74.34± 13.16*	0.96± 0.12*
Sevoflurane group（C,n=12）	56.23± 10.38*&	2.75± 0.34*&

Note: Compared with Sham group, *$P<0.05$; compared with CIRI group, &$P<0.05$.

2.3 大鼠海马 S100β 和 PGRN 表达情况

脑缺血再灌注损伤组和七氟醚组大鼠海马 S100β 和 PGRN 的表达较假手术组有显著上调，其中七氟醚组 S100β 表达较脑缺血再灌注损伤组显著降低，而 PGRN 表达显著升高（$P<0.05$），见表 3。

表 3 大鼠海马 S100β 和 PGRN 的表达水平($\bar{x}\pm s$, μg/L)

Table 3 Comparison of the expression levels of S100β and PGRN in each group($\bar{x}\pm s$, μg/L)

Groups	S100β	PGRN
Sham operation group（A,n=12）	0.14± 0.02	0.17± 0.01
CIRI group（B,n=12）	0.73± 0.11*	0.61± 0.04*
Sevoflurane group（C,n=12）	0.52± 0.06*&	0.82± 0.26*&

Note: Compared with Sham group, *$P<0.05$; compared with CIRI group, &$P<0.05$.

3 讨论

脑缺血再灌注损伤是动脉粥样硬化、脑缺血缺氧类心脑血管患者在治疗过程中脑组织血流量突然改变，血流再灌注，导致血管压力骤增，甚至破裂，对脑组织及中枢神经系统造成严重损伤[19]。CIRI 在急性心肌梗死、脑血栓等严重创伤性心脑血管疾病发展过程中均有参与，目前缺血性脑卒中约占脑卒中亚型的 85 %，我国每年死于缺血性脑卒中的人口约为 160 万[20]，已成为死亡和致残的主要原因之一。大量研究表明[21,22]，大脑海马区对脑缺血再灌注最为敏感，大鼠全脑缺血 1 d，海马 CA1神经元坏死率为 30 %，缺血 4 d，神经元坏死率急剧增加至 96 %。因此，作为与学习记忆功能密切相关的区域，大脑海马区在脑缺血再灌注损伤时会严重影响患者的认知功能。2000 年流行病学调查结果显示[23]，我国 70~90 岁的老年人当中，轻度认知功能障碍患者约占 10.46 %，2010 年人口调查报告显示，60 岁以上人口占总人口 13.3 %，估算患有认知功能障碍的人数在 3000 万以上。研究发现[24,25]影响患者认知功能的因素由多种，如炎症反应、血管性损伤等，诱导因素多种多样，其中最主要的因素为手术刺激，产生术后认知功能损伤，一般为持续数月的记忆功能、理解能力下降，严重者丧失生活能力发展为永久性认知障碍。七氟醚[26]作为临床常用的吸入麻醉药，具有出良好的脑组织保护作用。S100β 是一种细胞内钙结合蛋白家族 S100 蛋白的成员之一，在中枢神经系统中表达程度较高，当细胞受损时 S100β 蛋白诱导机体产生炎症反应，促使细胞功能性损伤进一步加重。颗粒蛋白前体（PGRN）对细胞的作用较为广泛，具有促进细胞增殖、营养、抗炎等多种功能，在神经系统中表达主

要分布在海马齿状回的锥体细胞层[27],脑缺血损伤后在脑内小胶质细胞与星形胶质细胞表达明显上调，研究表明[28],PGRN可通过抑制炎性反应、减轻血脑屏障的破坏发挥脑缺血损伤后的神经保护作用。因此,本研究通过探索七氟醚预处理对CIRI大鼠认知功能及海马S100β和PGRN蛋白表达水平的影响，为进一步探索七氟醚作用机制提供实验证据。

本研究结果显示,脑缺血再灌注损伤组和七氟醚组大鼠神经功能缺损评分显著高于假手术组,说明造模成功,七氟醚预处理组评分较脑缺血再灌注组有明显降低,说明从临床症状表现上看,七氟醚预处理组大鼠出现相应症状明显少于CIRI组，表现出明显的脑损伤保护作用,与Du R等[29]研究类似,该学者表明七氟醚可能通过降低心肌耗氧量,抑制炎症因子、激活线粒体信号传导通路、减少血管性损伤发挥脑组织的保护作用，减少患者的术后认知功能障碍的发生,其具体分子机制有待探索。Morris水迷宫实验结果表明,CIRI组和七氟醚组大鼠与假手术组相比逃逸潜伏期明显延长,表明造模后大鼠学习记忆功能较造模前显著退化，与CIRI组相比七氟醚组逃逸潜伏期显著缩短,说明七氟醚预处理组大鼠记忆功能比CIRI组强,进一步证实了七氟醚对大鼠脑损伤造成的认知障碍有明显缓解。与薛杭[30]等学者的研究类似,该学者评价七氟烷后处理对缺血缺氧性脑损伤新生大鼠远期脑白质髓鞘碱性蛋白(MBP)表达的影响,与对照组比较,缺血缺氧性脑损伤组逃避潜伏期延长,目标象限停留时间缩短,穿越原平台次数减少,与缺血缺氧性脑损伤组比较,缺血缺氧性脑损伤组＋七氟烷后处组逃避潜伏期缩短,目标象限停留时间延长,穿越原平台次数增多,表明七氟烷后处理减轻缺血缺氧性脑损伤新生大鼠远期认知功能障碍。本研究CIRI组和七氟醚组大鼠海马S100β和PGRN的表达较假手术组有显著上调,表明上述两种蛋白与脑损伤程度密切相关,与Jia D等[31]的研究类似,该学者发现缺血再灌注大鼠的缺血核心区与半暗带PGRN表达显著升高;同时Liu Ming等[32]的研究也发现PGRN在学习记忆过程中也有参与,PGRN基因敲除小鼠表现出明显的记忆功能下降，而本研究中七氟醚组PGRN表达显著升高,说明PGRN与学习记忆功能有明显的正相关关系,PGRN表达水平越高记忆功能越强。本研究通过基础研究,探究了七氟醚显著提高对脑缺血再灌注损伤大鼠的学习认知能力，其机制可能与S100β和PGRN在大鼠海马区细胞的表达水平存在明显相关关系,该结果为进一步探索七氟醚对脑缺血再灌注损伤大鼠认知功能影响的分子机制提供了实验参考依据,值得借鉴。

综上,七氟醚对脑缺血再灌注损伤大鼠进行预处理能显著提高大鼠的学习认知能力，其作用机制与S100β和PGRN在大鼠海马区细胞的表达水平存在明显相关关系,S100β蛋白表达越少,大鼠认知功能越强,PGRN表达水平越高记忆功能越强。

参 考 文 献(References)

[1] Fu-jiang Wang, Zuo-yan Sun, Rui-lin Li, et al. Protection of Salvianolate Lyophilized Injection combined with Xueshuantong Injection (Lyophilized) against focal cerebral ischemia/reperfusion injury in rats through suppression of inflammatory response [J]. Chinese herbal medicine, 2018, 10(1): 46-53

[2] Wang Yahui, Yao Xiaofeng, Zhang Dongya. microRNA-21 Confers Neuroprotection Against Cerebral Ischemia-Reperfusion Injury and Alleviates Blood-Brain Barrier Disruption in Rats via the MAPK Signaling Pathway [J]. J Molecular Neuroscience: MN, 2018, 65 (1): 43-53

[3] Fu-jiang WANG, Shao-xia WANG, Li-juan CHAI, et al. Xueshuantong injection (lyophilized) combined with salvianolate lyophilized injection protects against focal cerebral ischemia/reperfusion injury in rats through attenuation of oxidative stress [J]. Chinese Journal of Pharmacology, 2018, 39(6): 998-1011

[4] Xiao Lan, Xin Zhang, Guo-ping Zhou, et al. Electroacupuncture reduces apoptotic index and inhibits p38 mitogen-activated protein kinase signaling pathway in the hippocampus of rats with cerebral ischemia/reperfusion injury[J]. Research on nerve regeneration in China, 2017, 12(3): 409-416

[5] Shilei Wang, Junchao Liu, Qingxian Chang, et al. Neuroprotective effects of cromakalim on cerebral ischemia-reperfusion injury in rats Correlation with hippocampal metabotropic glutamate receptor 1 alpha and glutamate transporter 1[J]. Research on nerve regeneration in China, 2019, 5(9): 678-682

[6] Chen Su-hui, Sun Hua, Zhang Ya-min, et al.Effects of Acupuncture at Baihui (GV 20) and Zusanli (ST 36) on Peripheral Serum Expression of MicroRNA 124, Laminin and Integrin β1 in Rats with Cerebral Ischemia Reperfusion Injury [J]. Chinese Journal of Combined Medicine, 2017, 22(1): 49-55

[7] Qu Xiao-yu, Zhang Yue-ming, Tao Li-na, et al. XingNaoJing injections protect against cerebral ischemia/reperfusion injury and alleviate blood-brain barrier disruption in rats, through an underlying mechanism of NLRP3 inflammasomes suppression[J]. Natural medicines in China, 2019, 17(7): 498-505

[8] Ahmadi-Eslamloo, Hossein, Dehghani, Gholam Abbas, Moosavi, Seyed Mostafa Shid. Long-term treatment of diabetic rats with vanadyl sulfate or insulin attenuate acute focal cerebral ischemia/reperfusion injury via their antiglycemic effect [J]. Metabolic brain disease, 2018, 33(1): 225-235

[9] Lin, Ruhui, Lin, Yukun, Tao, Jing, et al. Electroacupuncture ameliorates learning and memory in rats with cerebral ischemia-reperfusion injury by inhibiting oxidative stress and promoting p-CREB expression in the hippocampus [J]. Molecular medicine reports, 2017, 12(5 Pt.A): 6807-6814

[10] Chen, Weijia, Wei, Shengnan, Yu, Yang, et al. Pretreatment of rats with increased bioavailable berberine attenuates cerebral ischemia-reperfusion injury via down regulation of adenosine-5' monophosphate kinase activity[J]. European Journal of Pharmacology: An International Journal, 2016, 779: 80-90

[11] Yuan Min, Ge Mingyue, Yin Jiangwen, et al. Isoflurane post-conditioning down-regulates expression of aquaporin 4 in rats with cerebral ischemia/reperfusion injury and is possibly related to bone morphogenetic protein 4/Smad1/5/8 signaling pathway [J]. Biomedicine & pharmacotherapy, 2018, 97: 429-438

[12] Wang G, Zhu J, Kang C, et al. Effect of basic fibroblast growth factor on behavior capability and neurons in the dentate gyrus of rats with atherosclerosis after cerebral ischemia reperfusion injury[J]. Biomedical Engineering: Applications [J]. Basis and Communications, 2019,

25(2): 1350025-1-1350025-10

[13] Zhu Jun-de, Wang Jun-jie, Ge Guo, et al. Effects of Noggin-Transfected Neural Stem Cells on Neural Functional Recovery and Underlying Mechanism in Rats with Cerebral Ischemia Reperfusion Injury [J]. Journal of stroke and cerebrovascular diseases, 2017, 26 (7): 1547-1559

[14] Yu Zhao, Shengtao Yao, Shijun Wang. Neural stem cell transplantation in the hippocampus of rats with cerebral ischemia/reperfusion injury Activation of the phosphatidylinositol-3 kinase/Akt pathway and increased brain-derived neurotrophic factor expression [J]. Research on nerve regeneration in China, 2018, 05(21): 1605-1610

[15] Tian Xiaocui, An Ruidi, Luo Yujie, et al. Tamibarotene Improves Hippocampus Injury Induced by Focal Cerebral Ischemia-Reperfusion via Modulating PI3K/Akt Pathway in Rats [J]. Journal of stroke and cerebrovascular diseases, 2019, 28(7): 1832-1840

[16] Yu Zhonghai, Cai Min, Zhang Wen, et al. Neuroprotective effects of Tongxinluo on focal cerebral ischemia and reperfusion injury in rats associated with the activation of the MEK1/2/ERK1/2/p90RSK signaling pathway[J]. Brain research, 2018, 1685: 9-18

[17] Qi Wenqian, Zhou Fangfang, Li Shuai, et al. Remote ischemic postconditioning protects ischemic brain from injury in rats with focal cerebral ischemia/reperfusion associated with suppression of TLR4 and NF-kappa B expression[J]. Neuroreport, 2016, 27(7): 469-475

[18] Shuang Jiang, Haina Zhang, Min Qian, et al. Effects of ginsenoside CK pretreatment on oxidative stress and inflammation in rats with cerebral ischemia/reperfusion injury[J]. Biotechnology & Biotechnological Equipment, 2018, 32(6): 1606-1612

[19] Dong Yanru, Bao Cuifen, Yu Jingwei, et al. Receptor-interacting protein kinase 3-mediated programmed cell necrosis in rats subjected to focal cerebral ischemia-reperfusion injury[J]. Molecular medicine reports, 2019, 14(1 Pt.A): 728-736

[20] Jinting He, Haiqi Li, Gaofeng Li, et al. Hyperoside protects against cerebral ischemia-reperfusion injury by alleviating oxidative stress, inflammation and apoptosis in rats [J]. Biotechnology & Biotechnological Equipment, 2019, 33(1): 798-806

[21] Zhong Pei, Raymond Tak Fai Cheung. Pretreatment with melatonin exerts anti-inflammatory effects against ischemia/reperfusion injury in a rat middle cerebral artery occlusion stroke model [J]. Journal of Pineal Research, 2015, 37(2): 85-91

[22] Jingping Shi, Jingde Dong, Jie Lu, et al. Effects of chronic peripheral pretreatment with an angiotensin Ⅱ type-1 receptor blocker on apoptosisrelated molecules in rats with cerebral ischemia/reperfusion injury [J]. Research on nerve regeneration in China, 2019, 05 (15): 1150-1155

[23] Ya-Nan Tang, Gao-Feng Zhang, Huai-Long Chen, et al. Selective brain hypothermiainduced neuroprotection against focal cerebral ischemia/reperfusion injury is associated with Fis1 inhibition [J]. Research on nerve regeneration in China, 2020, 15(5): 903-911

[24] Giacoppo, Sabrina, Galuppo, Maria, Iori, Renato, et al.(R-S)-glucoraphanin purified from Tuscan black kale and bioactivated with myrosinase enzyme protects against cerebral ischemia/reperfusion injury in rats[J]. Fitoterapia, 2014, 99: 166-177

[25] Yong Wang, Yan Shen, Hai-ping Lin, et al. Large-conductance Ca^{2+}-activated K^{+} channel involvement in suppression of cerebral ischemia/reperfusion injury after electroacupuncture atShuigou (GV26) acupoint in rats[J]. Research on nerve regeneration in China, 2018, 11 (6): 957-962

[26] Li-Jun Ge, Shou-Yan Fan, Jie-Hong Yang, et al. Pharmacokinetic and pharmacodyna-mic analysis of ferulic acid-puerarin-astragaloside in combination with neuroprotective in cerebral ischemia/reperfusion injury in rats [J]. Asia Pacific Journal of Tropical Biomedicine, 2017, (4): 299-304

[27] Xiao Xing-Jun, Qu, You-Yang, Liu, Yu, et al. The Protective Effect of Epoxyeicosatrie noic Acids on Cerebral Ischemia/Reperfusion Injury is Associated with PI3K/Akt Pathway and ATP-Sensitive Potassium Channels[J]. Neurochemical research, 2017, 40(1): 1-14

[28] Li Jun-Jie, Zou Zhi-Yao, Liu Jia, et al. Biliverdin administration ameliorates cerebral ischemia reperfusion injury in rats and is associated with proinflammatory factor downregulation [J]. Experimental and therapeutic medicine, 2017, 14(1 Pt.B): 671-679

[29] Du R, Zhou X,Yang D, et al. (Z)-7,4'-Dimethoxy-6-hydroxy-aurone-4-O-β-glucopyranoside alleviates cerebral ischemia-reperfusion injury in rats associating with the regulation of JAK1/STAT1 signaling pathway [J]. Human & Experimental Toxicology, 2020, 39 (11): 1507-1517

[30] 薛杭, 吴子怡, 赵平. 七氟醚后处理对缺血缺氧性脑损伤新生大鼠远期脑白质 MBP 表达的影响 [J]. 中华麻醉学杂志, 2020, 40(9): 1132-1135

[31] Jia D, Han B, Yang S, et al. Anemonin alleviates nerve injury after cerebral ischemia and reperfusion (I/R) in rats by improving antioxidant activities and inhibiting apoptosis pathway[J]. Journal of Molecular Neuroscience: MN, 2014, 53(2): 271-279

[32] Liu Ming, Shen Jie, Zou Fan, et al. Effect of ulinastatin on the permeability of the blood-brain barrier on rats with global cerebral ischemia/reperfusion injury as assessed by MRI [J]. Biomedicine & pharmacotherapy =: Biomedecine & pharmacotherapie, 2017, 85: 412-417