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银杏内酯对大鼠急性脊髓损伤保护作用的实验研究*

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摘要 目的:探讨银杏内酯对大鼠急性脊髓损伤的保护作用。**方法:**选取健康成年正常 SD 大鼠 54 只,分正常组、损伤组和银杏内酯治疗组;采用改良 Allen's 打击法制作脊髓损伤动物模型,分别在伤后 6 h、12 h、24 h、72 h 处死动物,采用免疫组织化学方法结合图像分析技术观测 NF-κB 和 COX-2 在脊髓腰段的表达情况。**结果:**脊髓神经功能评定显示银杏内酯治疗组大鼠神经功能较单纯损伤组有所改善;正常脊髓前角内 NF-κB 和 COX-2 有一定的基础表达。脊髓损伤后 6 h 脊髓神经元的胞浆及胞核内 NF-κ B 和 COX-2 均先后表达上升,24 h 达高峰,72 h 仍维持在较高水平;而给予银杏内酯治疗后,各时间点 NF-κB 和 COX-2 的表达上调幅度均降低。**结论:**急性脊髓损伤后,银杏内酯可通过控制 NF-κB 和 COX-2 的表达上调的幅度而抑制炎症反应,对脊髓受损神经元起一定的保护作用。

关键词:银杏内酯;脊髓损伤;NF-κB;COX-2

中图分类号:Q95-3,R641,R338.3 **文献标识码:**A **文章编号:**1673-6273(2014)26-5027-04

Experimental Study of Protective Effect of Ginkgolide in the Acute Spinal Cord Injury in Rats*

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ABSTRACT Objective: To investigate the effects of ginkgolide in experimental spinal cord injury (SCI) in rats. **Methods:** 54 healthy adult SD rats were distributed into normal group, injured group and ginkgolide treatment group. The model of was established by Allen's method at the level of L3-L5 with a moderate degree of damage. Ginkgolide was administered intraperitoneally immediately after operation in the ginkgolide treatment group. Rats were survived for 6, 12, 24 and 72 hours after operation. The expression and distribution of NF-κB and COX-2 in the lumber spinal cord were detected by immunohistochemical method and image analysis technique. **Results:** Nervous function of spinal cord in the ginkgolide treatment group was better than that in injured group after SCI in rats. NF-κB and COX-2 were expressed in normal anterior horn of spinal cord. The expression of NF-κB and COX-2 in neurons of spinal cord both began to up-regulate at 6 h, peaked at 24 h after SCI in order, still higher than normal level at 72 h . Up-regulated degree of expression in ginkgolide treatment group was lower than that in injured group after SCI in rats. **Conclusions:** Ginkgolide can inhibit the inflammatory reaction to protect injured neurons of spinal cord through the control of up-regulated degree of expression of NF-κB and COX-2 in the spinal cord after acute SCI in rats.

Key words: Ginkgolide; Spinal cord injury; NF-κB; COX-2

Chinese Library Classification(CLC): Q95-3, R641, R338.3 Document code: A

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前言

银杏内酯(Ginkgolide)是银杏植物中提取的天然产物,为强特异性的血小板活化因子拮抗剂,具有抑制血栓形成、清除自由基、抑制炎症反应、扩张脑血管及增加血流量等作用[1-5]。大量研究表明银杏内酯可减轻脑缺血损伤的不同区域的神经元的损伤和凋亡、保护脑神经元[5,6]。但银杏内酯对脊髓损伤后的炎症反应及脊髓神经元的保护作用鲜有文献报道。本实验采用大鼠急性脊髓损伤模型,研究银杏内酯对 NF-κB 和 COX-2 表达的影响,为探讨银杏内酯对受损脊髓神经元保护作用的研究提供实验依据。

1 材料与方法

1.1 实验动物分组、模型制备、给药、主要试剂与仪器

成年健康 SD 大鼠 54 只, 由湖南中医药大学动物学部提供,雌雄不限,重 200 g 左右,随机分为正常组(n=6)、损伤组

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(n=24)和银杏内酯治疗组(n=24)。SD 大鼠经 2 % 戊巴比妥钠腹膜腔注射麻醉(40 mg/kg)后,俯卧位固定,术前术野脱毛,以 L3-L5 棘突为中心做正中纵行切口,手术咬除 L3-L5 的棘突及相应双侧椎板,显露长约 2.0 cm 的硬膜囊;在细玻璃管的引导下用 10 g 圆柱状金属棒从 5 cm 高处垂直坠下,打击硬膜囊损伤脊髓;术中见大鼠尾巴痉挛摆动、躯体及双侧后肢回缩扑动,约 1 min 后见双后肢瘫痪;实验过程中无动物死亡。造模成功后 30 min 银杏内酯治疗组腹腔注射银杏内酯 10 mg·kg/d,各实验动物又根据术后存活时间随机分为 6 h、12 h、24 h、72 h 组(各组 n=6)后处死。

多克隆兔抗 NF-κB p50 抗体(武汉博士德生物工程有限公司),多克隆羊抗 COX-2(Transduction Laboratory),生物素化兔抗羊 IgG、生物素化羊抗兔 IgG 和辣根酶标记链酶卵白素(均购自 Santa Cruz 公司),DAB (Sigma 公司), 图像分析软件(Motic Advanced3.2),显微摄像系统(Motic,德国)。

1.2 脊髓神经功能评价

Tarlov 评分 0 级,无后肢活动,不能负重;1 级,后肢偶有活动,不能负重;2 级,后肢有力或频繁活动,不能负重;3 级,后肢能支持体重, 且可走 1- 2 步;4 级, 可行走且仅有轻度障碍;5 级,能正常行走。

1.3 取材、组织化学及图像数据分析

组织标本取材、SABC 法 NF-κB 和 COX-2 免疫组织化学染色、图像分析与统计学处理均参照课题组文献进行[7]。

2 结果

2.1 脊髓神经功能评定

急性脊髓损伤后,肌张力降低,大鼠后肢瘫软,在损伤平面以下出现各反射不同程度减弱或消失, 需进行人工排尿按压。银杏内酯治疗组的大鼠神经功能比单纯损伤组有所改善(见表 1)。

表 1 大鼠脊髓损伤后脊髓神经功能评定

Table 1 The assessment of nervous function of spinal cord after spinal cord injury in rats (Mean ± SD, n=6)

Injured time	Tarlov score	
	Injured group	Ginkgolide treatment group
6 h	0.29± 0.46	0.33± 0.48
12 h	0.67± 0.59	0.89± 0.16
24 h	1.33± 0.49	2.17± 0.58*
72 h	2.50± 0.67	3.42± 0.51*

注:*:vs 损伤组 P< 0.05。

Note: *:vs injured group P< 0.05.

2.2 NF-κB 在脊髓神经元的表达

过氧化物酶 -DAB 显色显示正常大鼠脊髓前角可见大量大小不等的 NF-κB 免疫阳性神经元, 免疫阳性产物呈均质状分布于胞浆,偶可见 NF-κB 胞核染色的活化细胞(图 1-A)。损伤组 6 h NF-κB 在脊髓前角神经元的胞浆和胞核免疫阳性产物表达增加,24 h 达高峰,72 h 仍维持在较高水平(图 1-B、C)。给予银杏内酯治疗后 6 h~ 24 h 神经元胞浆和胞核免疫阳性产物的表达亦逐渐增强,但升高的幅度仍比损伤组低(图 1-D),72 h 仍高于正常。NF-κB 免疫阳性灰度值测定显示:损伤组各时间点与正常组比较均有显著统计学差异(P<0.05);术后 24 h 银杏内酯治疗组与损伤组相比有统计学意义(P<0.05),实验所测数据(见图 2)。

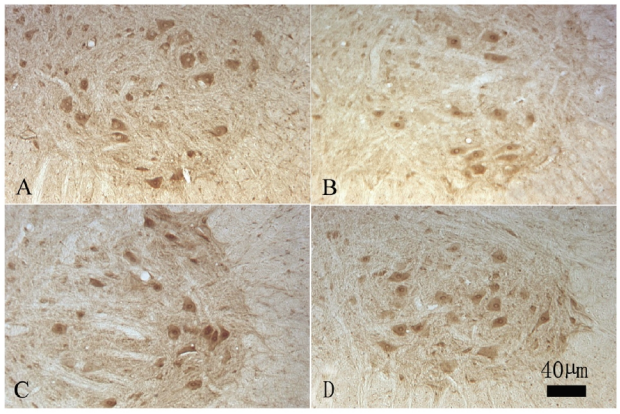

图 1 大鼠脊髓前角 NF-κB 的免疫组织化学

A 为正常对照组,B、C 分别为损伤组 6 h 和 24 h,D 为银杏内酯治疗组 24 h

Fig.1 Immunohistochemistry of NF-κB in anterior horn of spinal cord of rats

A:normal group; B and C: 6 h and 24 h groups of spinal cord injury; D: 24 h group of bilobalide therapy

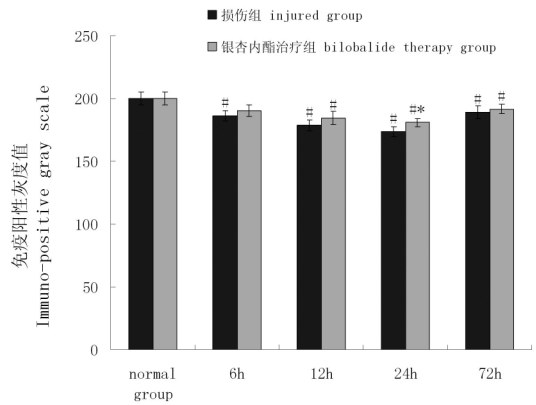

图 2 大鼠脊髓前角 NF-κB 的免疫阳性灰度值测定

Fig. 2 The NF-κB immuno-positive gray scale in anterior horn of spinal cord in rats

注:*:vs 损伤组 P< 0.05; #:vs 正常组 P< 0.05。

Note: *:vs injured group P< 0.05; #: vs normal group P< 0.05.

2.3 COX-2 在脊髓神经元的表达

正常大鼠 COX-2 免疫阳性产物在脊髓灰质神经元呈低水平表达 (图 3-A); 损伤后 6 h~ 24 h 脊髓 COX-2 表达逐渐增加,灰质各板层可见免疫阳性产物,主要分布于脊髓后角各板层神经元和前角运动神经元的胞浆和胞核(图 3-B、C)。银杏内酯治疗组 COX-2 于 6 h~ 24 h 的表达逐渐增强,但升高的幅度仍比损伤组低(图 3-D)。COX-2 免疫染色灰度值测量显示:术后 12 h、24 h 损伤组与正常组比较有显著统计学差异 (P< 0.05); 术后 24 h 银杏内酯治疗组与损伤组相比有统计学意义(P<0.05),实验所测数据(见图 4)。

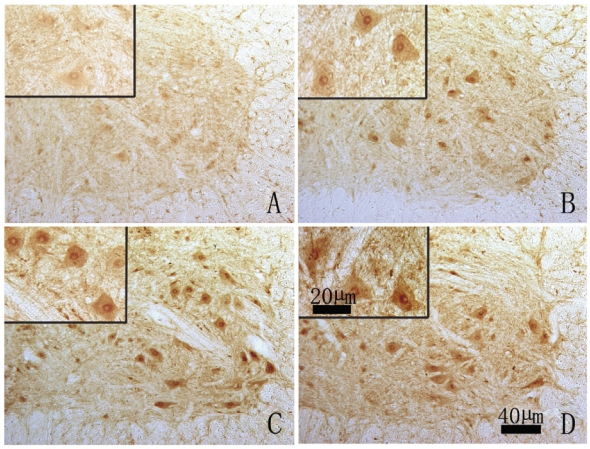

图 3 大鼠脊髓前角 COX-2 的免疫组织化学

A 为正常对照组，B、C 分别为损伤组 6 h 和 24 h，D 为银杏内酯治疗组 24 h

Fig. 3 Immunohistochemistry of COX-2 in anterior horn of spinal cord of rats

A:normal group; B and C: 6 h and 24 h groups of spinal cord injury; D: 24 h group of bilobalide therapy

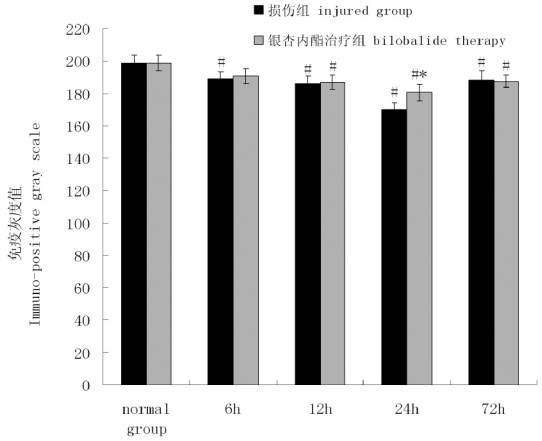

图 4 大鼠脊髓前角 COX-2 的免疫阳性灰度值测定

Fig.4 The COX-2 immuno-positive gray scale in anterior horn of spinal cord in rats

注：*:vs 损伤组 P＜0.05；#:vs 正常组 P＜0.05。

Note: *:vs spinal cord injury group P＜0.05; #: vs normal group P＜0.05.

3 讨论

急性脊髓损伤破坏血脊髓屏障，损伤区血管断裂，血管活性物质释放，致血管舒缩功能失调及通透性增加，进而引起损伤区缺血、缺氧和血管源性组织水肿[8]。脊髓损伤后 24 h 内，中性粒细胞、巨噬细胞、小胶质细胞等进入损伤区，均可释放大量对损伤修复具有双向调节作用的、可介导炎症和免疫反应的细胞因子[9,10]。炎症反应的复杂细胞因子网络中，NF-κB 的激活是一个中心环节[11]。NF-κB 是一类广泛存在于真核生物的关键性核转录因子，包括 p50、p52、p65/RelA 等，在胞浆以同源或异源二聚体的非活性形式与其抑制蛋白 Iκ Bs 形成复合物。缺血缺氧、创伤等可致 NF-κB 与 IκB 分离，NF-κB 被快速激活并移至核内与靶基因启动子区域 κB 基序结合，导致下游炎症因子蛋白（如 IL-1、TNF-α、COX-2 和 iNOS 等）的表达，参与免疫炎症反应、细胞凋亡和增殖等多种生物学过程[12-14]。

NF-κB 参与急性脊髓损伤后的炎症反应，对损伤后脊髓神经元的存活、再生修复、神经变性和神经病理性疼痛起重要的调节作用[12,15]。本课题组研究证实脊髓损伤后 NF-κB 在受损脊髓节段神经元内活化明显增加，24 h 达高峰，表明 NF-κB 被激活，可能在转录水平发挥调节作用，这与近年来的报道相似[15]。我们在给予银杏内酯治疗后，损伤部位的脊髓神经元的 NF-κB 的表达上调幅度降低；推测银杏内酯可能通过抗血小板活化因子的作用、降低血脊髓屏障通透性、减少脊髓缺血损伤区中性粒细胞的浸润、减少炎症介质的释放、避免细胞受低氧的伤害作用等方式抑制了急性脊髓损伤后级联的炎症反应的 NF-κB 激活[16]。

COX 为一种膜结合蛋白，是花生四稀酸转化为前列腺素过程中的限速酶，至少有两种异构体，即 COX-1 和 COX-2，COX-2 在脊髓损伤后的炎症反应中参与介导细胞毒性作用，与细胞膜的破坏有关[17]。本实验发现脊髓损伤后早期 COX-2 在脊髓内的表达随时间明显上调，24 h 内表达快速升高，之后升高较慢，72 h 仍维持在较高水平，这与近年来有关脊髓损伤后的 COX-2 表达变化结果相似[18]。脊髓损伤后 NF-κB 和 COX-2 的表达上调，可促使脊髓神经元释放谷氨酸，增加谷氨酸的兴奋性神经毒性[19]，产生炎症细胞因子、反应性氧物质和自由基导致细胞死亡[20]。而我们在给予银杏内酯治疗后，损伤部位的脊髓神经元的 COX-2 的表达上调幅度降低。我们推测，由于银杏内酯作为强特异性的血小板活化因子拮抗剂，抑制了脊髓损伤区缺血缺氧和组织水肿、抑制炎症反应和参与清除自由基，起到保护脊髓损伤区神经元的作用[21]。

综上所述，脊髓损伤早期 NF-κB 和 COX-2 的表达上调参与了损伤后的炎症反应；银杏内酯通过抑制炎症反应对脊髓受损神经元起一定的保护作用，但其保护神经元的具体机制还有待于进一步研究。

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