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·论　著·

二甲基亚砜对轴突退行性病变的保护作用

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[摘要]　**目的**：探讨二甲基亚砜(dimethylsulfoxide，DMSO)对轴突退行性病变的影响。**方法**：DMSO溶断组：用DMSO 10 μl溶断处理原代培养的大鼠颈上神经节细胞使胞体与轴突分离，并分别设阳性对照组(用过量表达 Wld^S 蛋白的重组HSV病毒感染细胞，剔除胞体)、空白对照组和DMSO对照组(分别将10 μl PBS和DMSO加入培养基，切断轴突并去除胞体)。分别于轴突与胞体分离即刻(0 h)及分离后4、8、12、24 h用4%多聚甲醛固定细胞，用抗神经特异的微管蛋白抗体做免疫荧光染色，观察轴突微管结构变化。收集轴突蛋白作免疫印迹分析，观察神经纤维(neurofilament)的降解情况。**结果**：DMSO溶断组在12 h时轴突的结构和形态没有明显改变，处理24 h后有轻微的轴突退行性病变；微管结构的崩解速度明显减缓，至12 h微管结构仍然能够维持完整；去除胞体后12 h，仍然能够检测到NF160神经元纤维蛋白。这种变化与阳性对照组非常相似，而空白对照组和DMSO对照组未见明显保护轴突退行性病变的作用。**结论**：局部高浓度的DMSO能够延缓轴突的退行性病变，提示DMSO可能用于神经退行性疾病的辅助治疗。

[关键词]　轴突；神经变性疾病；Wld^S 蛋白；二甲基亚砜；微管；神经纤维
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Protective effect of dimethylsulfoxide on axon degeneration

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[**ABSTRACT**]　**Objective**: To investigate the protective effect of dimethylsulfoxide (DMSO) on axon degeneration. **Methods**: Cultured rat superior cervical ganglia were treated with DMSO (100%, 10 μl) per well to disconnect axons from the cell bodies. SCGs in DMSO control group were treated with a mixture of DMSO (10 μl) and medium (2 ml) per well; in positive control group were transfected with herpes simplex virus over-expressing Wld^S protein and the cell body was eliminated; and in blank control group were treated with 10 μl PBS. The separated axons were fixed with 4% poly formaldehyde at 0, 4, 8, 12 and 24 h after treatment with DMSO for immunostaining with specific antibody to microtubulin. Thus, the changes of axonal structure were investigated. The axonal protein was collected and the degeneration of neurofilament was detected by Western Blotting. **Results**: In DMSO disconnected group, the axonal morphology and structure showed no obvious change at 12 h post disconnection, but slight degeneration was observed at 24 h post disconnection. The degradation of microtubulin was obviously slowed down and their axonal structures maintained intact 12 h later. The neurofilament could be detected 12 h after disconnection. The above changes in disconnected group were similar to those in positive control group. No obvious protective effects on axonal degeneration were observed in blank and DMSO control groups. **Conclusion**: Local high concentration of DMSO can delay axonal degeneration, which indicates that DMSO can be used for adjuvant treatment of neurodegenerative diseases.

[**KEY WORDS**]　axons; neurodegenerative diseases; Wld^S protein; dimethylsulfoxide; microtubules; nerve fibers
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神经退行性疾病的发病率极高，最近调查发现中国65岁以上的老年人中帕金森病(Parkinson disease)的发病率高达1.7%[1,2]。这些疾病给家庭和社会带来了沉重的负担，因此寻找治疗这些疾病的有效治疗方法是目前生物医学研究中的一个热点和重点。轴突的退行性病变是很多神经退行性病变过程中的早期事件[3]，通过保护或者延缓轴突的退行性病变来治疗神经退行性疾病已经逐渐成为一个新兴的研究领域。原代培养神经细胞的轴突被切断以后，轴突会很快发生退行性病变，这个模型广泛地用于神经退行性病变的研究[4]。突变小鼠C57BL/Wld^S 由于一个融合基因的过量表达，能够显著延缓轴突退行性病变[5]。中枢神经系统来源的背根神经节细胞中过量表达 Wld^S 能够明显保护轴突的退行性病变[6]。这些结果提示 Wld^S 蛋白对轴突退行病变的保护作用具有普遍性。二甲基亚砜(dimethylsulfoxide，DMSO)在一定的条件下能够促进细胞膜

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的融合[7],并且能够促进损伤的背根神经节细胞膜修复,从而对损伤的神经细胞起保护作用[8]。在低温低钙条件下,DMSO 也能够促进中枢神经细胞的细胞膜修复[9]。这些结果表明 DMSO 促进受损神经细胞膜修复从而保护神经细胞,可能具有普遍性。本研究以 Wld^S 蛋白作为阳性对照,观察了 DMSO 对培养颈上神经节细胞轴突远端神经退行性病变的影响,旨在探讨 DMSO 对轴突退行性病变的保护作用。

1 材料和方法

1.1 仪器和试剂 CX31 奥林巴斯倒置临床检测显微镜,IX70-142 奥林巴斯荧光倒置显微镜,BIO-RAD 公司 Western 印迹电泳系统、转膜装置,CO_2 细胞培养设备。DMSO 购自 Fisher 公司,Dulbecco's Modified Eagle Medium 培养基、Nutrient Mixture Ham's F12 培养基和 Leibovitz's-15 培养基为 Gibco 公司产品,鼠源 Cy3 抗体[Cy™3-conjugated AffiniPure Goat Anti-Mouse IgG(H+L)]购自 Jackson ImmunoResearch Laboratpries Inc.,NF160 抗体(mouse anti-neurofilament-160 000,NFM)购自 Zymed 公司,神经细胞特异性微管蛋白抗体(monoclonal antibody against neuronal class Ⅲ β-tubulin)购自 Cripinc 公司,鼠源神经生长因子(nerve growth factor 7S,NGF7S)购自 Invitrogen 公司。其他常用生化试剂均为国产分析纯。

1.2 大鼠颈上神经节细胞培养 依据文献方法[10],将出生 3 d 的 SD 大鼠(购自上海斯莱克实验动物有限公司)取出颈上神经节,剪成大小均匀的组织块作原代神经细胞的组织培养。在 5%CO_2 培养箱中 37℃培养至第 5 天更换新鲜培养液备用。

1.3 实验分组 (1)DMSO 溶断组:吸取 10 μl DMSO,在显微镜下从距胞体约为轴突长度的 1/3 处轻轻贴住培养皿底部,沿垂直轴突将 DMSO 轻轻推出,防止 DMSO 波及远端轴突。这样局部的 DMSO 导致轴突和胞体分离,我们将这一处理过程称为 DMSO 溶断(DMSO disconnection)。(2)阴性对照:设空白对照组和 DMSO 对照组,分别将 10 μl PBS 和 DMSO 加入培养基中,混合均匀,观察轴突退行性病变。为了避免胞体对轴突的影响,用刀片切断轴突并去除胞体。(3)阳性对照组:采用携带 Wld^S 基因的 HSV 病毒感染原代培养的神经细胞作为阳性对照,依据文献方法[11]略做改动。将 Wld^S 基因的编码区插入 HSV 病毒载体构建病毒包装质粒备用。用带 Wld^S 基因的 HSV 包装质粒转染 2-2 细胞。24 h 后加入辅助包装病毒(helper virus),待 90%以上的细胞变圆,回收病毒。用第 1 次回收的病毒(P0 代病毒)重新感染 2-2 细胞,同样的步骤重复 2 次回收 P3 代病毒。P3 代 HSV 病毒感染颈上神经节细胞,待病毒充分感染后剔除胞体。

1.4 结果的观察 分别于轴突与胞体分离即刻(0 h)及分离后 4、8、12、24 h 用 4%多聚甲醛固定细胞,用抗神经特异的微管蛋白抗体做免疫荧光染色,观察轴突微管结构变化。依据文献[11]方法随机计算大约 200 根以上轴突,统计发生退行性病变的轴突占总数的比例。重复 3 次,得到不同时间轴突病变率的柱形图。收集轴突蛋白作免疫印迹分析,观察神经纤维(neurofilament)的降解情况。

1.5 统计学处理 应用 SPSS 10.0 软件进行 ANOVA 方差分析。

2 结 果

2.1 DMSO 对轴突退行性病变的影响 空白对照组和 DMSO 对照组结果基本类似,当轴突与胞体分离 8 h 后轴突远端部分发生典型的退行性病变:完整的轴突结构自末梢开始逐渐断裂,12 h 后轴突的形态遭到严重破坏,24 h 后几乎见不到完整的轴突结构。阳性对照组在轴突切断后 12 h,甚至 24 h,轴突仍然能够保持形态和结构的完整性。DMSO 溶断组在 12 h 时轴突的结构和形态没有明显改变,处理 24 h 后有轻微的轴突退行性病变。详见图 1。

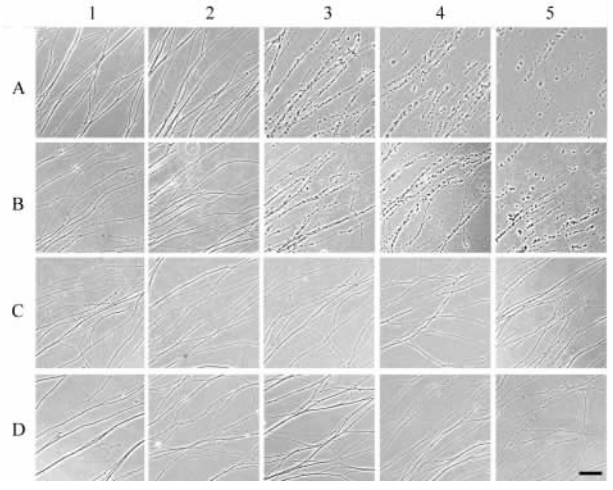

图 1 各组轴突退行性病变的形态学改变

Fig 1 Morphological changes of axon degeneration delayed in different groups

A: Blank control; B: DMSO control; C: DMSO disconnection; D: Positive control group; 1-5: 0, 4, 8, 12, 24 h post-axotomy, respectively. Scale bar, 40 μm

统计学分析显示,DMSO 溶断组与阳性对照组在

轴突分离后 12 h，只有 20%左右的轴突发生退行性病变，而 DMSO 对照组及空白对照组 97%以上均发生了退行性病变（$P<0.01$，图 2），这一结果进一步表明 DMSO 溶断处理能够延缓轴突的退行性病变。

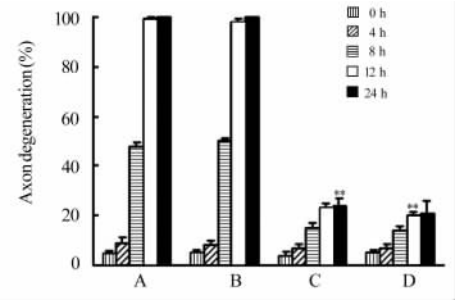

图 2　各组发生退行性病变的轴突所占比例

Fig 2　Quantification of axon degeneration in different groups

A：Blank control；B：DMSO control；C：DMSO disconnection；D：Positive control；** $P<0.01$ *vs* bland control and DMSO control group at post-axontomy 12 h；$n>200$

2.2　DMSO 对轴突微管结构的影响　空白对照组和 DMSO 对照组，轴突与胞体分离之后微管结构逐渐开始破坏，出现断裂现象，至 12 h 和 24 h 出现微管结构的大量碎片，表明微管结构大部分已经崩解而不足以维持轴突的结构和形态。DMSO 溶断组和阳性对照组，微管结构的崩解速度明显减缓，至 12 h 微管结构仍然能够维持完整（图 3）。

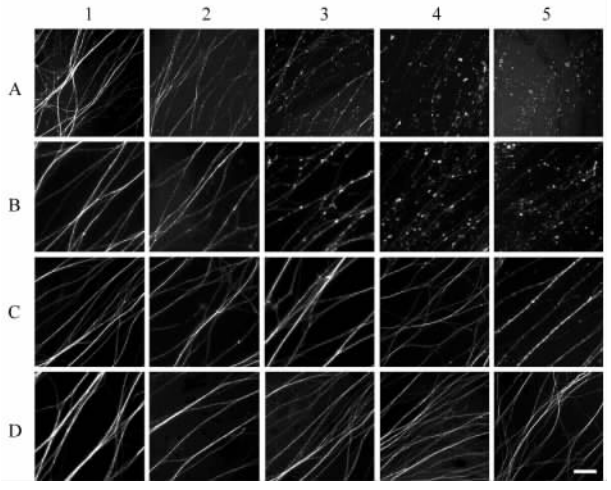

图 3　各组微管结构的变化

Fig 3　Changes of axon structure in different groups

A：Blank control；B：DMSO control；C：DMSO disconnection；D：Positive control group；1-5：0，4，8，12，24 h post-axotomy，respectively. Scale bar，40 μm

用抗神经元纤维的抗体作免疫印迹检测发现，轴突与胞体分离后 12 h，DMSO 对照组免疫印迹已经不能检测到相对分子质量为 160 000 的神经元纤维，而 DMSO 溶断组和阳性对照组仍然能够检测到这种神经元纤维蛋白（图 4）。这表明 DMSO 溶断和过量表达 Wld^S 基因均能延缓神经元纤维的降解。

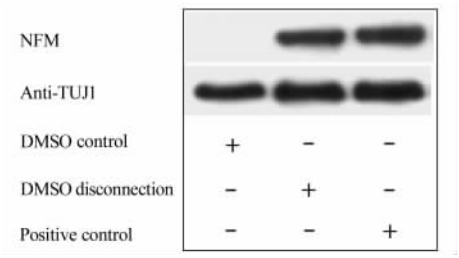

图 4　各组神经元纤维免疫印迹分析

Fig 4　Western blotting assay of neurofilaments in different groups

NFM：Mouse anti-neurofilament-16 000；Anti-TUJ1：Monoclonal antibody against neuronal class Ⅲ β-tubulin

3　讨　论

轴突的退行性病变是很多神经退行性病变的早期重要病理学特征之一[6]。当轴突发生退行性病变时，作为轴突主要结构蛋白的微管蛋白逐渐破坏，但微管蛋白的数量并没有发生明显的变化，因此可以通过检测微管蛋白作为内参。轴突退行性病变的另外一个检测指标是神经原纤维的降解，NF160 的降解是其中重要指标之一[12]。免疫印迹检测结果表明 DMSO 溶断以及过量表达 Wld^S 基因均能稳定微管的结构和形态，并延缓 NF160 的降解。而不论是空白对照组还是 DMSO 对照组均未见类似作用。这些结果在一定程度上表明局部高浓度的 DMSO 能够延缓轴突的退行性病变。

我们以前的研究表明在轴突发生退行性病变的早期，有蛋白酶体的激活，抑制蛋白酶体的活性能够保护轴突的退行性病变[11]。轴突 ATP 水平、钙离子的浓度以及轴突膜的完整性在轴突退行性病变中起重要作用。神经外伤或者疾病状况导致轴突膜的损伤可能引起一些离子或者蛋白质因子的损失，诱导轴突退行性病变，如我们实验中的对照组用刀切断轴突诱导的轴突退行性病变。DMSO 能够破坏与细胞膜相互作用的肌动蛋白从而破坏细胞膜下的细胞骨架网络结构，增加膜的流动性从而促进细胞膜的修复[13]；DMSO 也可能通过影响膜融合促进细胞膜的修复[14]。我们的实验结果表明低浓度的 DMSO 对照组（均匀加入 10 μl DMSO）与空白对照组类似，对轴突退行性病变未见任何保护作用。因此我

们推断用 DMSO 对轴突做溶断处理,可能在断裂位置的局部形成一个 DMSO 由高到低的浓度梯度,高浓度的 DMSO 导致轴突的断裂,但当 DMSO 浓度逐渐降低,达到一定浓度时可能促进轴突膜的融合从而保护轴突的退行性病变。另外,如前所述蛋白酶体活性的激活以及 Erk 信号通路也涉及到轴突退行性病变这一过程[15]。DMSO 是否也通过影响这些因素延缓轴突的退行性病变,有待于进一步研究。

[参 考 文 献]

[1] Muchowshi PJ. Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones [J]? Neuron, 2002, 35:9-12.

[2] Zhang ZX, Roman GC, Hong Z, et al. Parkinson's disease in China: prevalence in Beijing, Xi'an, and Shanghai [J]. Lancet, 2005, 365:595-597.

[3] Raff MC, Whitmore AV, Finn JT. Axonal self-destruction and neurodegeneration [J]. Science, 2002, 296:868-871.

[4] Waller A. Experiments on the section of glossopharyngeal and hypoglossal nerves of the frog and observations of the alternative produced therapy in the structure of their primitive fibers [J]. Phlios Trans R Soc Lond, 1850, 140:423-429.

[5] Mack TG, Reiner M, Beirowski B, et al. Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene [J]. Nat Neurosci, 2001, 4:1199-1206.

[6] Araki T, Sasaki Y, Milbrandt J. Increased nuclear NAD^+ biosynthesis and SIRT1 activation prevent axonal degeneration [J]. Science, 2004, 305:1010-1013.

[7] Yu ZW, Quinn PJ. The modulation of membrane structure and stability by dimethyl sulphoxide [J]. Mol Membr Biol, 1998, 15:59-68.

[8] Howard MJ, David G, Barrett JN. Resealing of transected myelinated mammalian axons *in vivo*: Evidence for involvement of calpain [J]. Neuroscience, 1999, 93:807-815.

[9] Shi R, Qiao X, Emerson N, et al. Dimethylsulfoxide enhances CNS neuronal plasma membrane resealing after injury in low temperature or low calcium [J]. J Neurocytol, 2001, 30:829-839.

[10] Zhai Q, Wang J, Kim A, et al. Involvement of Ubiquitin-Proteasome system in the early stages of Wallerian degeneration [J]. Neuron, 2003, 39:217-225.

[11] Wagstaff MJ, Collaco-Moraes Y, Smith J, et al. Protection of neuronal cells from apoptosis by Hsp27 delivered with a herpes simplex virus-based vector [J]. J Biol Chem, 1999, 274:5061-5069.

[12] Finn JT, Weil M, Archer F, et al. Evidence that Wallerian degeneration and localized axon degeneration induced by local neurotrophin deprivation do not involve caspases [J]. J Neurosci, 2000, 20:1333-1341.

[13] Wincler B, Forscher P, Mellman I. A diffusion barrier maintains distribution of membrane proteins in polarized neurons [J]. Nature, 1999, 397:698-701.

[14] Miyake K, Mcneil PL, Suzuki K, et al. An actine barrier to resealing [J]. J Cell Sci, 2001, 114:3487-3494.

[15] MacInnis BL, Campenot RB. Regulation of Wallerian degeneration and nerve growth factor withdrawal-induced pruning of axons of sympathetic neurons by the proteasome and the MEK/Erk pathway [J]. Mol Cell Neurosci, 2005, 28: 430-439.

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Rapid nongenomic inhibitory effects of glucocorticoids on phagocytosis and superoxide anion production by macrophages

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[**ABSTRACT**] Traditionally, steroid hormone effects have been described as a result of the modulation of nuclear transcription, thus triggering genomic events that are responsible for physiological effects. Despite early observations of rapid steroid effects that were incompatible with this theory, nongenomic steroid effects have been widely recognized only recently. However, the nongenomic effect of glucocorticoid (GC) on anti-inflammation and immunosuppression has not been reported. Macrophages play important roles in inflammation and the immune response. The present experiment selected macrophages as experimental cells to explore the nongenomic effects and possible mechanisms of GCs on phagocytosis and superoxide anion production. Phagocytosis by macrophages was detected by the neutral red uptake assay. The superoxide anions were measured by cytochrome C reduction assay. It was found that both 10^{-4} and 10^{-5} mol/L corticosterone (CORT) rapidly inhibited uptake of neutral red by macrophages in less than 30 min, and the inhibition by the former was stronger than that of the latter. CORT (10^{-4} to 10^{-10} mol/L) rapidly inhibited superoxide anion production by macrophages in less than 30 min. The above-mentioned effects were insensitive to the GC-receptor antagonist mifepristone (RU486) and the translation inhibitor actidione. CORT coupled to bovine serum albumin (BSA-CORT) was able to mimic the rapid inhibitory effects of CORT. The results indicated that CORT could rapidly inhibit phagocytosis and superoxide anion production by mouse peritoneal macrophages in vitro in less than 30 min by a rapid, nongenomic mechanism, which contributes to the anti-inflammatory and immunosuppressive actions of GCs. These data shed a new light on the clinical application of GCs.

[Steroids, 2005, 70: 55-61]