.实验研究.

黄芪甲苷对大鼠蛛网膜下腔出血后脑血管痉挛的影响

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【摘要】**目的** 探讨黄芪甲苷对大鼠蛛网膜下腔出血(SAH)后脑血管痉挛(CVS)的作用。**方法** 将成年雄性SD大鼠40只随机分为正常组、SAH组、二甲基亚砜组(DMSO,腹腔注射等体积0.1% DMSO)和黄芪甲苷组(腹腔注射用0.1% DMSO助溶的黄芪甲苷溶悬液,2 mg/ml),每组10只。采用血管内刺破法制作SAH模型。HE染色观察基底动脉形态改变;免疫组化染色分析基底动脉Toll样受体4(TLR4)、核转录因子kappa B(NF-κB)的表达。**结果** 与正常组相比,SAH组和DMSO组基底动脉管壁明显增厚(P<0.05),而管腔横截面积明显缩小(P<0.05);SAH组和DMSO组无明显差异(P>0.05)。黄芪甲苷组基底动脉管壁厚度较DMSO组明显变薄(P<0.05),管腔横截面积较DMSO组明显增大(P<0.05)。与正常组相比,SAH组和DMSO组基底动脉TLR4、NF-κB阳性率明显增高(P<0.05),而SAH组和DMSO组无明显差异(P>0.05)。黄芪甲苷组基底动脉TLR4、NF-κB阳性率较DMSO组明显下降(P<0.05)。**结论** 黄芪甲苷可能通过干预TLR4、NF-κB介导的炎症信号通路来缓解大鼠SAH后CVS。

【关键词】蛛网膜下腔出血;脑血管痉挛;Toll样受体4;核转录因子-κB;黄芪甲苷;大鼠

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Effects of Astragaloside Ⅳ on delayed cerebral vasospasm after subarachnoid hemorrhage

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【**Abstract**】 **Objective** To explore the effect of Astragaloside Ⅳ (AS-Ⅳ) on delayed cerebral vasospasm after subarachnoid hemorrhage (SAH) and its mechanism. **Methods** Forty male SD rats were randomly were divided into 4 groups of 10 animals each, i.e. normal, SAH+AS-Ⅳ, SAH+ dimothyl sulfoxide (DMSO) and SAH groups. The SAH model was made by endovascularly puncturing the internal carotid artery. The rats were given daily by intraperitoneal injection of AS-Ⅳ suspension in SAH+AS-Ⅳ group 30 minutes after the establishment of the model, equal volume of 0.1% DMSO in SAH+DMSO group, and equal volume of saline in the normal and SAH groups. The morphological changes in the basilar arteries were observed by a microscope, the expressions of TLR4 and NF-κB p65 in the basilar arteries were determined by immunohistochemical technique, and the plasma levels of TNF-α and IL-6 were determined by enzyme-linked immunosorbent assay. **Results** The thickness of the basilar artery wall was significantly thinner in the normal group than that in SAH+AS-Ⅳ group (P<0 .05), which was significantly thinner than that those in SAH and SAH+DMSO groups (P<0.05). The area of transverse section of the basilar artery was significantly bigger in the normal group than that in SAH+AS-Ⅳ group (P<0.05), which was significantly bigger than those in SAH and SAH+DMSO groups (P<0.05). The levels of TLR4 and NF-κB p65 expressions in the basilar arteries were significantly lower in the normal group than those in SAH+AS-Ⅳ group (P<0.05), which were significantly lower in SAH and SAH+DMSO groups (P<0.05). The plasma levels of TNF-α and IL-6 were significantly lower in the normal group than those in SAH+AS-Ⅳ group (P<0.05), which were significantly lower in SAH and SAH+DMSO groups (P<0.05). **Conclusion** AS-Ⅳ can alleviate the delayed cerebral vasospasm after SAH via inhibiting TLR4 and NF-κB-mediated inflammatory signaling pathways.

【**Key words**】 Astragaloside ; Subarachnoid hemorrhage; Delayed cerebral vasospasm ; TLR4; NF-κBⅣ

脑血管痉挛(cerebral vasospasm,CVS)是动脉瘤性蛛网膜下腔出血(subarachnoid hemorrhage,SAH)患者致残、死亡的主要原因之一。目前认为,Toll样受体4(toll-like receptor 4, TLR4)、核转录因子kappa B(nuclear factor kappa B,NF-κB)介导的免疫炎症级联反应是CVS发生的重要机制之一。黄芪甲苷能有效抑制TLR4、NF-κB介导的免疫炎症,对保护心肌具有作用[1]。本研究探讨黄芪甲苷对大鼠SAH后CVS的作用。

1 材料与方法

1.1 *动物及分组* 将雄性SD大鼠40只(体重为250~300 g,由我国军事医学科学院实验动物中心提供)随机分为正常组、黄芪甲苷组、二甲基亚砜

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(dimothyl sulfoxide, DMSO)组和SAH组，每组10只。

1.2 试剂 黄芪甲苷购自天津马克生物技术有限公司；TLR4单克隆抗体、p65多克隆抗体购自美国Abcam公司；免疫组化试剂盒、DAB显色剂盒购自北京中杉金桥生物技术有限公司。

1.3 SAH模型制作 采用血管内刺破法制作SAH模型[2]。5%水合氯醛(8 ml/kg)麻醉大鼠，暴露颈总动脉、颈内动脉、颈外动脉。结扎颈外动脉远心端，动脉夹阻断颈总动脉、颈内动脉，于颈外动脉结扎处近心端剪一小口，插入直径为0.2 mm尼龙线，剪断颈外动脉并牵拉使其与颈内动脉成一条直线，将尼龙线插入颈内动脉，有突破感后立即拔出尼龙线并结扎颈外动脉近心端，松开动脉夹，缝合切口并消毒。

1.4 药物配制与给药方法 将黄芪甲苷、生理盐水、0.1% DMSO配制成黄芪甲苷混悬液(2 mg/ml)，其中0.1% DMSO作为助溶剂。造模30 min后，黄芪甲苷组大鼠经腹腔注射黄芪甲苷混悬液(20 mg/kg)，DMSO组给予等体积0.1% DMSO；此后，每隔24 h执行上述操作，直至大鼠被处死。

1.5 取材及其检测方法

1.5.1 取材 SAH后5 d，4%多聚甲醛灌注固定，分离基底动脉连同脑干，后固定24 h后，常规石蜡包埋。

1.5.2 HE染色 将包埋好的石蜡块以层厚5 μm连续切片，HE染色，光镜下观察。使用ImagePro-Plus 6.0软件测量管壁厚度和横截面积。

1.5.3 免疫组化染色 将基底动脉石蜡切片脱蜡等常规处理后，按照免疫组化试剂盒中的说明书操作，DAB显色，苏木素复染，常规脱水、透明、封片。光镜下观察，每张切片选取3个视野(400倍)，分别分别计算TLR4、p65阳性率。

1.6 统计学分析 使用SPSS 19.0软件分析；计量资料以 $\bar{x}\pm s$ 表示，采用单因素方差分析和LSD检验；$P<0.05$ 表示差异有统计学意义。

2 结 果

2.1 基底动脉管壁厚度及管腔横截面积变化 与正常组相比，SAH组和DMSO组基底动脉管壁厚度明显增加($P<0.05$)，而管腔横截面积明显缩小($P<0.05$)；SAH组和DMSD组无明显差异($P>0.05$)。与DMSO组比，黄芪甲苷组基底动脉管壁厚度及管腔横截面积均明显改善。见表1、图1。

2.2 基底动脉TLR4、p65的表达变化 与正常组比，SAH组和DMSO组基底动脉TLR4、p65阳性率明显增高($P<0.05$)，而SAH组和DMSO组无明显差异($P>$

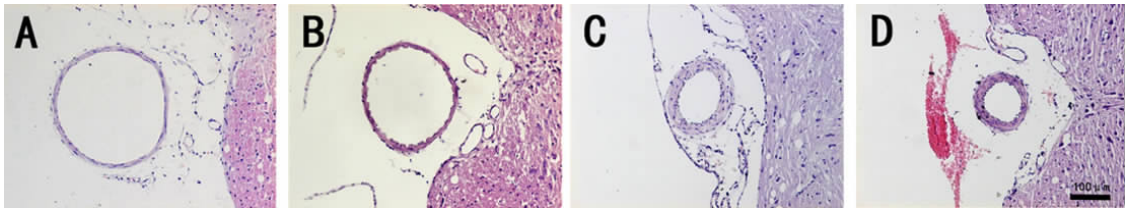

图1 各组大鼠基底动脉HE染色图(×100)

A. 正常组；B. 黄芪甲苷组；C. 二甲基亚砜组；D. 蛛网膜下腔出血组

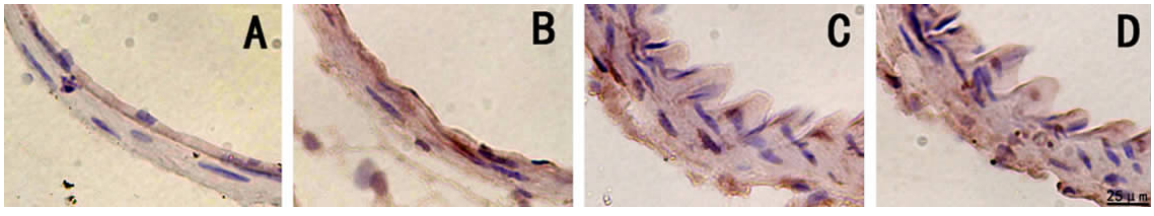

图2 各组大鼠基底动脉Toll样受体4免疫组化染色图(×400)

A. 正常组；B. 黄芪甲苷组；C. 二甲基亚砜组；D. 蛛网膜下腔出血组

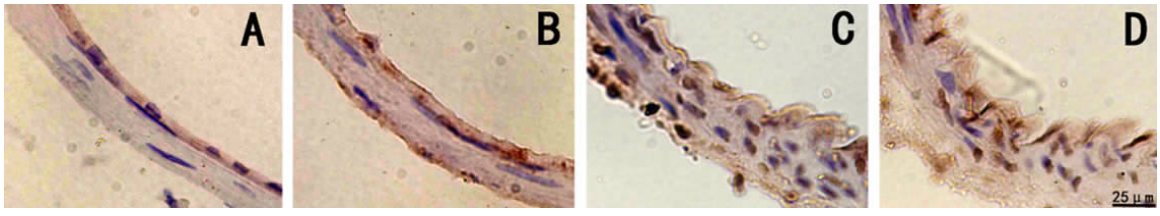

图3 各组大鼠基底动脉p65免疫组化染色图(×400)

A. 正常组；B. 黄芪甲苷组；C. 二甲基亚砜组；D. 蛛网膜下腔出血组

0.05）。与DMSO组比，黄芪甲苷组基底动脉TLR4、p65阳性率明显下降（P<0.05）。见表2和图2、3。

表1 各组大鼠基底动脉管壁厚度及管腔横截面积比较（$\bar{x}\pm s$）

组别	管壁厚度（μm）	管腔横截面积（μm²）
正常组	11.72±1.099	48 413±3 123
黄芪甲苷组	17.97±1.190*	30 281±2 791*
二甲基亚砜组	28.19±1.944*#	9 975±1 750*#
SAH组	29.18±1.949*#	8 996±1 620*#

注：与正常组相应值比，* P<0.05；与黄芪甲苷组相应值比，# P<0.05；SAH：蛛网膜下腔出血

表2 各组大鼠基底动脉TLR4、p65表达阳性率比较（$\bar{x}\pm s$）

组别	TLR4	p65
正常组	24.3%±4.6%	29.6%±4.%3
黄芪甲苷组	34.5%±4.2%*	46.1%±6.1%*
二甲基亚砜组	45.4%±6.3%*#	60.8%±9.4%*#
SAH组	48.%0±6.4%*#	61.9%±6.6%*#

注：与正常组相应值比，* P<0.05；与黄芪甲苷组相应值比，# P<0.05；TLR4：toll样受体4；SAH：蛛网膜下腔出血

3 讨 论

目前，CVS发生的具体机制尚不明确，CVS的治疗方法也不统一。虽然美国心脏协会SAH指南中，关于预防和治疗CVS的唯一一个Ⅰ级证据是使用尼莫地平，但尼莫地平对SAH患者总致残率和总死亡率没有明显改善[3,4]。为了寻找治疗CVS的新靶点，研究发现TLR4、NF-κB介导的免疫炎症级联反应具有潜在的应用价值。SAH后，血液进入蛛网膜下腔，红细胞溶血释放氧合血红蛋白，可刺激血管平滑肌细胞，导致TLR4表达显著增高[5]。也有研究发现兔SAH后TLR4也呈高表达状态，且与CVS的发展有关[6]。TLR4被激活后，主要通过髓样分化因子88依赖的信号通路进一步激活NF-κB，进而调节免疫炎症因子的释放[7]。

研究发现，黄芪甲苷具有多种药理活性作用，包括抗炎、免疫调节、抗凋亡及保护心血管等。在缺血再灌注导致的大鼠急性肾损伤模型中，黄芪甲苷可通过干预NF-κB介导的炎症反应从而达到保护肾脏的作用[8]。同样，在缺血再灌注导致的脑损伤模型中，黄芪甲苷可能通过调控基质金属蛋白酶9、水通道蛋白4及抑制中性粒细胞粘附分子等机制发挥着脑保护作用[9,10]。本研究结果显示黄芪甲苷可通过下调基底动脉血管壁TLR4、NF-κB表达，减轻炎症反应，从而改善大鼠基底动脉痉挛。

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