.实验研究.

慢性硬膜下血肿脂联素和基底膜蛋白多糖的表达

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【摘要】**目的** 探讨慢性硬膜下血肿脂联素(APN)、基底膜蛋白多糖(PL)的表达变化。**方法** 2019年5月至2020年5月钻孔引流术治疗CSDH共32例,所有病人入院后均服用阿托伐他汀钙片,直至复查CT示血肿完全吸收。术前、术后3 d、术后3周各取静脉血5 ml,术中采集血肿液5 ml,采用酶联免疫吸附法检测APN和PL的浓度。**结果** 血肿液APN浓度[(12.3±1.9)ng/ml]较外周血[(13.8±0.7)ng/ml]明显降低(P<0.05),而PL浓度[(10.9±4.3)ng/ml]较外周血[(7.6±2.3)ng/ml]明显增高(P<0.05)。术后3 d外周血APN浓度[(14.1±0.7)ng/ml]较术前无明显变化(P>0.05),术后3周外周血APN浓度[(15.3±0.8)ng/ml]较术前明显增高(P<0.05)。血肿液APN浓度与血肿液PL浓度呈明显负相关(r=−0.585,P<0.01);血肿液APN浓度与血肿量呈明显负相关(r=−0.486,P<0.01);血肿液PL浓度与血肿量呈明显正相关(r=0.557,P<0.01)。**结论** 本文结果提示APN和PL在CSDH发生、发展过程中具有一定作用,APN可作为CSDH阿托伐他汀治疗反应的评估指标。

【关键词】慢性硬膜下血肿;脂联素;基底膜蛋白多糖

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Expression of adiponectin and perlecan in chronic subdural hematoma

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【**Abstract**】 **Objective** To explore the expression of adiponectin (APN) and perlecan (PL) in the chronic subdural hematoma (CSDH). **Methods** The hemotoma and peripheral blood levels of APN and PL were detected in 32 patients with CSDH who underwent drilling drainage from May 2019 to May 2020 and received atorvastatin calcium tablets until the hemotomas were completely absorbed using the enzyme-linked immunosorbent assay. **Results** The APN concentration in hematoma [(12.3±1.9) ng/ml] was significantly lower that [(13.8±0.7) ng/ml] in peripheral blood before the operation (P<0.05), and PL concentration in hemotoma [(10.9±4.3) ng/ml] was significantly higher than that [(7.6±2.3) ng/ml] in peripheral blood before the operation (P<0.05). The APN concentration in peripheral blood 3 days after the surgery [(14.1±0.7) ng/ml; P>0.05] did not significantly changed and significantly increased 3 weeks after the surgery [(15.3±0.8) ng/ml; P<0.05] compared with before the surgery. The hematoma APN concentration was significantly negatively correlated with the hematoma PL concentration (r=0.585, P<0.01). The concentration of hematoma APN was significantly negatively correlated with the amount of hematoma (r=−0.486, P<0.01). The hematoma PL concentration was significantly positively correlated with the amount of hematoma (r=0.557, P<0.01). **Conclusions** Our results suggest that APN and PL have a certain role in the pathogenesis and development of CSDH, and the APN can be used as an assessment indicator of atorvastatin treatment for the CSDH.

【**Key words**】 Chronic subdural hematoma; Adiponectin; Perlecan

慢性硬膜下血肿(chronic subdural hematoma,CSDH)以老年男性居多,长期酗酒和服用抗凝药物是CSDH的易患因素[1]。目前,首选钻孔引流术治疗,但是术后复发率在9.2%~26.5%[2,3]。CSDH的发病机制仍不明确。文献报道,脂联素(adiponectin,APN)和基底膜蛋白多糖(perlecan,PL)在肥胖、脑卒中、心血管疾病及恶性肿瘤病人外周血中均表达异常[4,5]。本研究检测CSDH血肿液和外周血液APN、PL水平,分析它们在CSDH的发病过程中作用。

1 材料与方法

1.1 *研究对象* 2019年5月至2020年5月钻孔引流术治疗CSDH共32例,其中男21例,女11例;平均年龄(67.9±8.7)岁;血肿量(80.2±24.5)ml;6例双侧血肿,20例出现脑疝。纳入标准:①颅脑CT或MRI检查确

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诊为CSDH；②外伤病人在伤后3周以上出现呕吐、头痛、意识障碍；③所有病人都签署知情同意书。排除标准：①排除糖尿病、血液疾病、冠心病或服用抗凝药物的病人；②排除外伤导致的急性颅内出血；③严重的心脏及肝肾功能不全；④排除其他系统出血或感染的病人。

1.2 样本采集 术前取静脉血5 ml，术中在切开硬脑膜、打开血肿外膜时取血肿液5 ml，3 600转/min离心10 min分离血清，-80 ℃冻存。术后3 d、3周各取静脉血5 ml，离心分离血清。所有病人入院后均服用阿托伐他汀钙片，直至复查CT示血肿完全吸收。

1.3 检测方法 采用Neobioscience公司人Adiponectin ELISA试剂盒检测APN浓度，Boster公司人Perlecan ELISA试剂盒检测PL浓度。严格按照说明书操作，酶标仪测定450 nm吸光度，按所测值绘制标准曲线，并计算APN和PL浓度。

1.4 统计学分析 采用SPSS 20.0软件分析；计量资料用$\bar{x}\pm s$表示，采用t检验；采用Pearson相关系数分析相关性；P<0.05为差异有统计学意义。

2 结 果

2.1 CSDH血肿液APN、PL浓度变化 CSDH血肿液APN浓度[(12.3±1.9)ng/ml]较外周血[(13.8±0.7)ng/ml]明显降低(P<0.05)，而PL浓度[(10.9±4.3)ng/ml]较外周血[(7.6±2.3)ng/ml]明显增高(P<0.05)。

2.2 手术前后外周血APN浓度变化 术后3 d外周血APN浓度[(14.1±0.7)ng/ml]较术前无明显变化(P>0.05)，术后3周外周血APN浓度[(15.3±0.8)ng/ml]较术前明显增高(P<0.05)。

2.3 相关性分析 血肿液APN浓度与血肿液PL浓度呈明显负相关(r=-0.585，P<0.01)；血肿液APN浓度与血肿量呈明显负相关(r=-0.486，P<0.01)；血肿液PL浓度与血肿量呈明显正相关(r=0.557，P<0.01)。

3 讨 论

研究表明，CSDH病人凝血和纤溶反应存在严重的因子失衡。CSDH血肿液促炎性因子如血管内皮细胞生长因子(vascular endothelial growth factor，VEGF)、核转录因子-κB(nuclear factor-κB，NF-κB)、白介素-6(interleukin 6，IL-6)、缺氧诱导因子(hypoxia-inducible factor，HIF)等水平明显升高[6]。VEGF是最强烈的促血管生成因子之一，通过增加微血管通透性，使血浆内容物大量渗出，不断给新生血管的形成提供营养支持，诱导毛细血管生长，促进膜壁形成脆弱的血管网[7]。IL-6通过重新排列肌动蛋白丝和改变内皮细胞的形态来扩大内皮细胞间空隙，使血浆内容物更容易渗出，并与VEGF共同增大新生血肿膜毛细血管的通透性。缺氧时，HIF-1α大量表达，通过调节炎性因子激活NF-κB，活化的NF-κB产生免疫应激反应加重血管内皮损伤，与HIF-1α共同参与CSDH的发生及病理改变。APN的主要生物学效应是通过刺激APN受体，增加5′-腺苷酸和过氧化物酶体增殖物激活受体的活性[8]。此外，APN还可抑制NF-κB信号传导[9]，并可抑制脂多糖诱导的肿瘤坏死因子-α(tumor necrosis factor alpha，NTF-α)的表达[10]。这表明APN能够减轻炎症反应，起到负性免疫调节作用。本文结果显示CSDH血肿液APN浓度明显低于静脉血，提示血肿腔炎性反应活跃，APN浓度下降不能起到抑制炎症反应的作用。

PL是一种硫酸乙酰肝素蛋白多糖，是细胞外基质的组成成分之一，参与细胞增殖与粘附，在新生血管和血管内皮损伤中具有重要作用[11,12]。据报道，在未损伤的血管中，PL的表达水平较低，在慢性病变如多发性硬化、动脉粥样硬化等，PL的表达水平明显增高[13]。在正常血管组织中，PL可稳定血管基底膜结构；但在血管损伤期间，PL从受损的细胞外基质释放，通过调节成纤维细胞生长因子，促进血管生成和内皮细胞增殖[14]。除此之外，PL可以通过促进细胞黏附，或通过激活VEGF，间接参与纤维化形成[15]。由于VEGF可激活血肿外膜有丝分裂原活化蛋白激酶信号通路，修饰PL的硫酸乙酰肝素侧链，同样可调节促有丝分裂的生长因子的活性。本研究发现，血肿液PL浓度明显高于静脉血。因此，我们推测，CSDH血肿腔存在大量PL，促进局部炎症反应，导致TNF-α、VEGF、IL-6等大量分泌聚集，PL可协同这些炎性刺激因子，促进新鲜血管生成，提高纤溶亢进，而尚未发育成熟的血管结构缺损，细胞间缝隙较大，血浆容易渗出，从而引起血肿腔内不断出现凝血、纤溶、出血，使血肿逐渐增加[16]。

另外，阿托伐他汀作为调脂、抗动脉粥样硬化的药物应用于临床治疗CSDH，具有抗炎、神经保护和血管生成调节等作用[17]。本文结果显示服用阿托伐他汀治疗3周，静脉血APN浓度明显增高。这与既往报道一致[18]。研究显示，血浆APN水平可反映脑脊液APN水平，这为他汀类药物临床治疗CSDH提供了理论支持。Wang等[19]发现，阿托伐他汀可促进内皮祖细胞分化，不仅可以修复基底膜不完整的内皮细胞，还能参与损伤血管的再生。

由于无法取得术后残余的血肿液，不能检测术后血肿液APN含量。而APN具有抑制炎症因子和抗血管粥样硬化的作用；PL具有促炎性因子，促进纤溶及促进血管粥样硬化的作用[20]。本文相关性分析显示，血肿液APN与PL呈负相关，这提示两者在CSDH发生起促进或抑制作用。

总之，CSDH血肿液PL显著升高，APN显著降低，两者在CSDH发生、发展中具有一定作用，APN可作为CSDH阿托伐他汀治疗反应的评估指标。

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