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苦参素对实验性大鼠肝纤维化防治作用的研究

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Effect of Oxymatrine on experimental liver fibrosis

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Abstract

AIM:To study the effect of Oxymatrine on liver fibrosis in immunogenic liver fibrosis rat model.

METHODS:Rat liver fibrosis model was induced by human serum albumin (HSA), 60 Wistar rats were randomly divided into 5 groups, control group without any treatment,liver fibrosis model group,oxymatrine preventive group, oxymatrine therapeutic group,and cochicine therapeutic group.The pathological changes of liver were observed by HE and Von-Gieson staining.The expressions of mRNA and proteins of collagen I/III in liver were determined by in situ hybridization and immunohistochemistry.

RESULTS:The liver fibrosis degree and level of mRNA and proteins of collagen I/III in the liver were significantly reduced in the decreasing order in oxymatrine preventive group, oxymatrine therapeutic group, and cochicine therapeutic group.

CONCLUSION:Oxymatrine may inhibit hepatic inflammation and hepatic synthesis of collagen I/III,and thus prevent and inhibit hepatic fibrosis.

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摘要

目的:探讨苦参素对实验性大鼠肝纤维化的预防与治疗作用.

方法:采用人血清白蛋白免疫损伤 Wistar 大鼠造成肝纤维化模型，60 只 Wister 大鼠，分为 5 组:造模组、秋水仙碱治疗组、试验组 I（苦参素预防组）、试验组 II（苦参素治疗组），另设正常对照组.动态观察大鼠肝组织病例变化，采用 HE 染色观察肝组织改变及 Von-Gieson 胶原纤维特殊染色观察肝纤维化程度，并采用免疫组织化学染色及肝组织原位杂交检测 I 型、III 胶原含量及 I 型、III 型胶原 mRNA 的表达.

结果:试验组肝组织结构明显好转，纤维组织增生程度明显低于肝纤维化模型组；I 型、III 型胶原含量及 I 型、III 型胶原的 mRNA 表达均低于模型组免疫诱导型肝纤维化大鼠，其效果为试验组 I（苦参素预防组）效果最好，试验组 II（苦参素治疗组）次之，秋水仙碱治疗组再次之，三组均优于造模组.

结论:苦参素可减轻肝脏炎症活动、抑制肝内胶原合成，对实验性大鼠肝纤维化具有防治作用.

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0 引言

肝纤维化是慢性肝损伤向肝硬化发展的基础,是由于肝细胞外基质增生与降解失衡所至，其主要病理变化是肝细胞外基质在肝脏的过渡沉积[1-4].目前，人们普遍认为，此阶段是可逆的病理过程[5-8].目前国内外对于肝纤维化尚无理想的治疗方法，因此，寻找有效药物，阻断、延缓及逆转肝纤维化的发生和发展是急待解决的问题，是治疗肝硬化的关键.曾有文献[9-12]报道秋水仙碱具有抑制肝细胞外基质生成的作用，并曾应用于少数病例，但由于疗效不佳，毒副作用较大，临床应用受到限制.苦参素是从中药苦豆子和苦参根中提取的有效成分，具有抗炎、免疫调节、稳定细胞膜及阻断肝细胞凋亡等作用[13-17].有文献报道，苦参素临床应用具有抗纤维化作用[18,19]，本研究应用免疫损伤性大鼠肝纤维化模型，初步探讨了苦参素抗肝纤维化的作用机制, 以期为其临床应用提供可靠的理论依据.

1 材料和方法

1.1 大鼠肝纤维化模型的制备 Wister 大鼠♀，体重 140±20 g，60 只，清洁级（购自第四军医大学实验动物中心）.取 54 只 Wister 大鼠参照文献[20,21]应用

人血白蛋白免疫攻击方法制备免疫性肝纤维化大鼠模型(人血白蛋白，选自兰州生物制品研究所，不完全福氏佐剂系 sigma 公司产品).抗大鼠 IgG 单克隆抗体购自法国 Coulter 公司.另 6 只 Wister 大鼠设为正常对照组.

1.2 动物分组 试验组 I(10 只):尾静脉攻击同时，开始予苦参素（氧化苦参碱，博尔泰力注射液，由宁夏绿谷药业公司生产）50 mg/kg 腹腔内注射 1 次/d,维持 3 mo，造膜结束后存活 8 只；44 只实验攻击动物存活 29 只，按随机分组法随机分为 4 组：模型组（9 只):尾静脉攻击结束后不予任何其他干扰因素;治疗组（10 只):尾静脉攻击结束后予秋水仙碱（系昆明制药股份有限公司生产）0.28 mg/kg 灌胃治疗，6 次/wk，维持 3 mo；试验组 II（10 只)：尾静脉攻击结束后，予苦参素 50 mg/kg 腹腔内注射 1 次/d，维持 3 mo；正常对照组（6 只)：等量无菌生理盐水腹腔内注射,1 次/d，维持 3 mo.所有动物均于给药 3 mo 后处死，打开腹腔，取肝脏方叶小块组织置 10 % 中性甲醛固定，留作病理检查.

1.3 检测指标

1.3.1 病理学观察 常规 HE 染色观察肝组织学改变；Von Gieson 胶原纤维特殊颜色.

1.3.2 Ⅰ型、III型胶原的免疫组化染色 Ⅰ型、III型胶原免疫组化试剂盒购自武汉博士德生物工程有限公司，兔抗Ⅰ型、III型胶原单克隆抗体试剂编号分别为 BA0325 和 BA0326.具体方法按试剂盒说明书常规进行操作.

1.3.3 肝组织原位杂交 Ⅰ型、III型前胶原 mRNA 表达检测采用地高辛标记探针原位杂交法.Ⅰ型、III前胶原原位杂交试剂盒均购自武汉博士德生物工程有限公司，试剂编号分别为MK1171和MK1549.按试剂盒说明书常规进行操作.

1.3.4 图像分析与数据处理 免疫组化及杂交结果经计算机图像灰度扫描数字转换后，进行统计数字处理，各组间行 t 检验，以 P 值<0.05 判断有显著性差异.

2 结果

2.1 肝脏病理变化肉眼观察 正常大鼠肝脏颜色鲜红，表面光滑，质脆柔软.造模组大鼠肝脏体积缩小， 质地较韧，边缘较钝，颜色呈暗红色，表面粗糙.肝组织汇管区中可见纤维组织增生，同时，纤维组织于中央静脉周围增生并向肝小叶内延伸，但纤维间隔较纤细，汇管区及中央静脉周围可见炎细胞浸润，沿界板及其他部位有肝细胞浓染、溶解及小双核细胞.造模组 V-G 染色胶原纤维沿汇管区及中央静脉周围向肝小叶内延伸，交联成网状结构(图 1).试验组 I、试验组 II 与治疗组大鼠肝脏在色泽、质地、大小及表面光滑度方面较造模组均有明显改善.光镜检查可见肝脏组织内炎性细胞浸润、肝细胞坏死和纤维组织增生等方面均较造模组大鼠为轻，纤维隔较薄.

2.2 Ⅰ型、III型胶原肝组织免疫组化 免疫组化检测的阳性信号呈现为棕黄色颗粒状，正常肝脏Ⅰ型胶原阳性染色只见于汇管区和中央静脉管壁，III 型胶原主要存在于大血管周围；造模组肝脏中Ⅰ型、III型胶原广泛存在于纤维间隔及肝细胞内；试验组 I、试验组 II 与治疗组大鼠肝脏中Ⅰ型、III型胶原明显减少，纤维间隔较薄.将胶原免疫组化结果进行图像分析，表明试验组 I、试验组 II 与治疗组肝脏中Ⅰ型、III型胶原较造模组明显减少.杂交结果经计算机图像灰度扫描数字转换值见表 1.

2.3 肝组织原位杂交 原位杂交检测的阳性信号呈现为棕黄色颗粒状，分布在肝细胞质内，呈散在或弥漫性分布，未见细胞核着色(图 2).将Ⅰ型、III型前胶原 mRNA 表达结果进行图像分析，表明试验组 I、试验组 II 与治疗组肝脏Ⅰ型、III型前胶原 mRNA 表达较造模组明显减少.结果经计算机图像灰度扫描数字转换值见表 2.

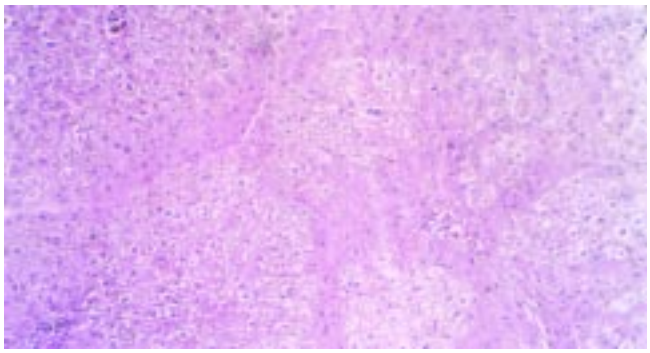

图 1 造模组大鼠肝组织 V-G 染色（×400）

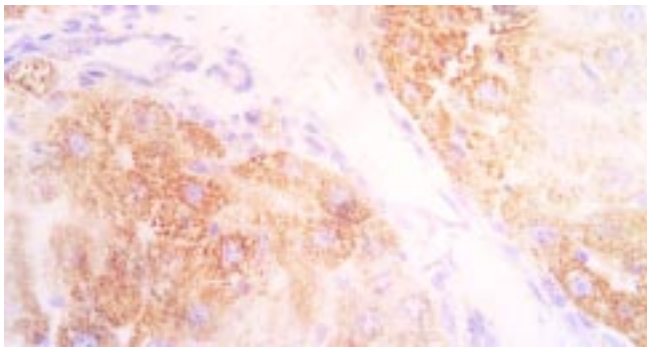

图 2 试验组大鼠肝组织中Ⅰ型前胶原 mRNA 原位杂交结果（×400）

表 1 各组大鼠肝脏组织Ⅰ、III型胶原免疫组化结果(灰度扫描数字转换值)

分组	*n*	Ⅰ型胶原	III 型胶原
造模组	9	476.11±160.32	676.67±182.81
试验组 I	8	162.88±40.63[bc]	380.88±77.38[bd]
试验组 II	10	226.1±77.7[b]	518.2±89.6[a]
治疗组	10	319±148.6[a]	579.1±136.88
对照组	6	68±10.57	99.67±23.67

[a]P <0.05vs 造模组比较;[b]P <0.01vs 造模组比较;

[c]P <0.05 vs 治疗组比较;[d]P <0.05 vs 治疗组比较.

表2 各组大鼠肝脏组织Ⅰ、Ⅲ型前胶原mRNA表达量(灰度扫描数字转换值)

分组	*n*	Ⅰ型前胶原mRNA	Ⅲ型前胶原mRNA
造模组	9	1 258.11±229.23	1 587.44±280.62
试验组Ⅰ	8	798.25±129.81[b]	867.38±134.31[bc]
试验组Ⅱ	10	947.1±250.12[a]	1143.6±238.32[b]
治疗组	10	959.2±244.64[a]	1 258.3±344.5[a]
对照组	6	228.17±50.56	235.83±70.89

[a]P <0.05vs 造模组比较;[b]P <0.01vs 造模组比较;
[c]P <0.05 vs 治疗组比较;[d]P <0.05 vs 治疗组比较.

3 讨论

多种病因所致的慢性肝损伤，由于肝细胞的变性坏死、纤维组织增生，最终可发展成肝硬变，因此，肝纤维化是慢性肝炎向肝硬变发展的必经阶段.肝纤维化是继发于肝脏炎症或损伤后组织修复过程中的代偿反应.目前认为，肝纤维化的发生机制主要是细胞外基质(EMC)的过度增生并在肝内异常沉积[22-30].细胞外基质包括胶原、糖蛋白及蛋白多糖等，其中胶原是最主要的成分.肝纤维化的形成过程主要取决于胶原的合成、沉积、降解和吸收的动态平衡.当胶原合成与沉积大于降解和吸收时，肝内胶原纤维增加，逐渐形成肝纤维化.胶原在正常肝组织中只占蛋白成分的5-10 %，但在肝硬化组织中可增高到50 %以上，而Ⅰ型胶原可占硬化肝脏胶原总量的60-70 %，Ⅲ型胶原可占硬化肝脏胶原总量的20-30 %[31-33]，因而Ⅰ型、Ⅲ型胶原可作为反应胶原代谢的重要参数[34-38]，观察Ⅰ型、Ⅲ型胶原合成的多少,可以判断抗纤维化药物的疗效[39,40].原位杂交是一项分子病理学技术，主要检测的是正在表达的基因[45,46]，定位性强，敏感性高，有利于对肝纤维化发病机制的研究；地高辛是近年应用较广的非同位素标记物，其敏感性高，具有特异性好，储存稳定等特点，同时避免了同位素的污染，易被人们接受使用[47-49].在肝纤维化的形成过程中，前胶原mRNA的增高先于或同步于胶原的增加，前胶原的转录过程是胶原合成的限速步骤.与组织学检测相比，前胶原mRNA的测定能更好地反映当时肝脏胶原合成的状况，并可提示肝纤维化的发展趋势[41-44].

目前,人们普遍认为,肝纤维化是可逆的病理过程[5-8].因此,寻找有效药物，阻断延缓及逆转肝纤维化的发生和发展是急待解决的问题.抗肝纤维化的治疗，国内外虽陆续有过一些报道，但因种种原因尚未找到十分理想的药物，众多学者仍在进行有益的探索.近些年来，国内学者报道以中药为主或中西药物联合应用治疗肝纤维化,动物实验和临床应用均显示出较好的苗头[47-55].苦参素(oxymatrine)又名氧化苦参碱,系由中药苦豆子中提取的生物碱类,大量研究表明,苦参素有抗HBV作用,能阻断肝细胞凋亡、稳定细胞膜、消除自由基、保护肝细胞,调节免疫功能及防治肝纤维化等作用[13-19].

本实验采用人血白蛋白注射所致免疫诱导性大鼠肝纤维化模型，用苦参素治疗大鼠肝纤维化.应用苦参素预防和治疗的大鼠，其肝表面色泽、质地较肝纤维化模型组明显改善，Ⅰ型、Ⅲ型前胶原mRNA均较造模组明显降低，治疗效果依次为：试验组Ⅰ> 试验组Ⅱ>治疗组>模型组.Ⅰ型、Ⅲ型胶原的免疫组化也显示同样的结果.表明苦参素能有效地抑制大鼠肝内Ⅰ型、Ⅲ型胶原的累积；降低肝纤维化大鼠肝内Ⅰ型、Ⅲ型前胶原mRAN的表达，因而推测苦参素可能通过抑制肝脏胶原合成而实现抗纤维化作用，也可通过抗炎、抗病毒、抑制免疫等环节起到抗肝纤维化作用.而其抑制成纤维细胞的增生及原胶原mRNA的表达可能是其抗肝纤维化的主要环节.另外，鉴于苦参素无明显毒副作用，有望成为很有前途的抗肝纤维化药物，其临床疗效还有待进一步深入研究.

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