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血清脯肽酶与肝损害*

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提 要 采用CCl_4致小鼠急、慢性肝损伤模型,平行动态观察了肝组织学改变与同期血清脯肽酶(PLD)和丙氨酸转氨酶(ALT)变化关系。急性肝损伤中,血清PLD和ALT活性均明显升高,且二者变化呈显著正相关($n=100$,$r=0.3853$,$P<0.01$)。PLD峰值期为注射CCl_4后16 h,与肝坏死最严重的时相一致。PLD峰值和恢复时间均早于ALT。肝硬化形成大致可分为胶原纤维增生(1~6周),肝硬化形成(6~9周)和肝硬化(9~12周)3个阶段。其间血清PLD活性第1周开始升高、3~6周达高峰,而后开始下降,9周后降至正常,而ALT无明显变化。结果表明,血清PLD活性测定是一项诊断急性肝损伤的敏感指标,除外急性肝损伤、PLD活性在某种程度能较好地反映进展性肝纤维化。

关键词 脯肽酶;丙氨酸转氨酶;肝纤维化;肝硬变;小鼠

中图法分类号 R575.2

脯肽酶(Prolidase,PLD)是体内广泛存在的一种二肽水解酶,富含于肝细胞,其血清水平变化与肝损伤程度及肝纤维化进展相关[1~4]。本文通过CCl_4致小鼠急、慢性肝损伤模型,平行动态观察肝组织学改变与同期血清PLD和丙氨酸转氨酶(ALT)水平变化的关系,评价血清PLD活性测定在肝病诊断中的意义。

1 材料与方法

1.1 动物分组及急、慢性肝损伤模型的建立

昆明小鼠250只,雌雄各半,8~10周龄,体重25~40 g,均由本校动物所提供。250只小鼠随机分为正常对照组(30只)、急性肝损伤组(100只)和慢性肝损伤组(120只)。急慢性肝损伤模型采用Myara介绍的方法通过腹腔内注射CCl_4建立[2]。CCl_4每次注射剂量为1.5 μl/g。急性肝损伤仅注射1次。根据注射后采集血和肝组织标本时间(1、3、6、8、16 h和1、2、3、4、7 d)分10组,每组10只。慢性肝损伤每周注射2次。根据每只小鼠注射的总周数(1、3、6、9、12周,不同,再分5组,120只小鼠仅88只完成实验,另32只中途死亡。。血及肝组织标本均在末次注射后96 h采集。血标本通过眼动脉采集,分离血清-40℃保存,3月内行PLD和ALT活性测定。肝组织标本在活杀动物后剖腹摘取,10%福尔马林液固定备用。

1.2 内容及方法

1.2.1 血清PLD活性测定 采用本室与成都制药九厂联合研制的市售商品试剂盒检测。

1.2.2 血清ALT活性测定 采用市售商品试剂盒与PLD同步检测。

1.2.3 肝脏病理检查 肝组织切片行HE染色和胶原纤维染色,观察肝组织损伤程度并参照Schvarcz标准[5]进行肝纤维化分级和评分。Ⅰ级(1分):肝组织无胶原纤维和成纤维细胞增生;Ⅱ级(2分):胶原纤维从汇管区或中央静脉区向外轻度延伸,伴成纤维细胞轻度增行;Ⅲ级(3分):胶原纤维延伸明显,形成假小叶雏形,伴成纤维细胞活跃增生;Ⅳ级(4分):纤维结缔组织在肝实质内广泛增生,分割成大小不等假小时,伴大量纤维母细胞增生。慢性肝损伤各组每只小鼠肝脏病理按上述标准分级评分,计算各组肝纤维化分级总积分(Tootal liverr fibrosis,Tf))和各组肝纤维化分级平均分(Means lives fibroosis,Mf)($Mf=\frac{Tf}{n}$)。

1.3 统计学处理

$\bar{x}$比较用t检验,测定值大于$\bar{x}+1.96s$为异常,计算异常率,异常率比较用χ^2检验,相关分析用相关检验。

2 结果

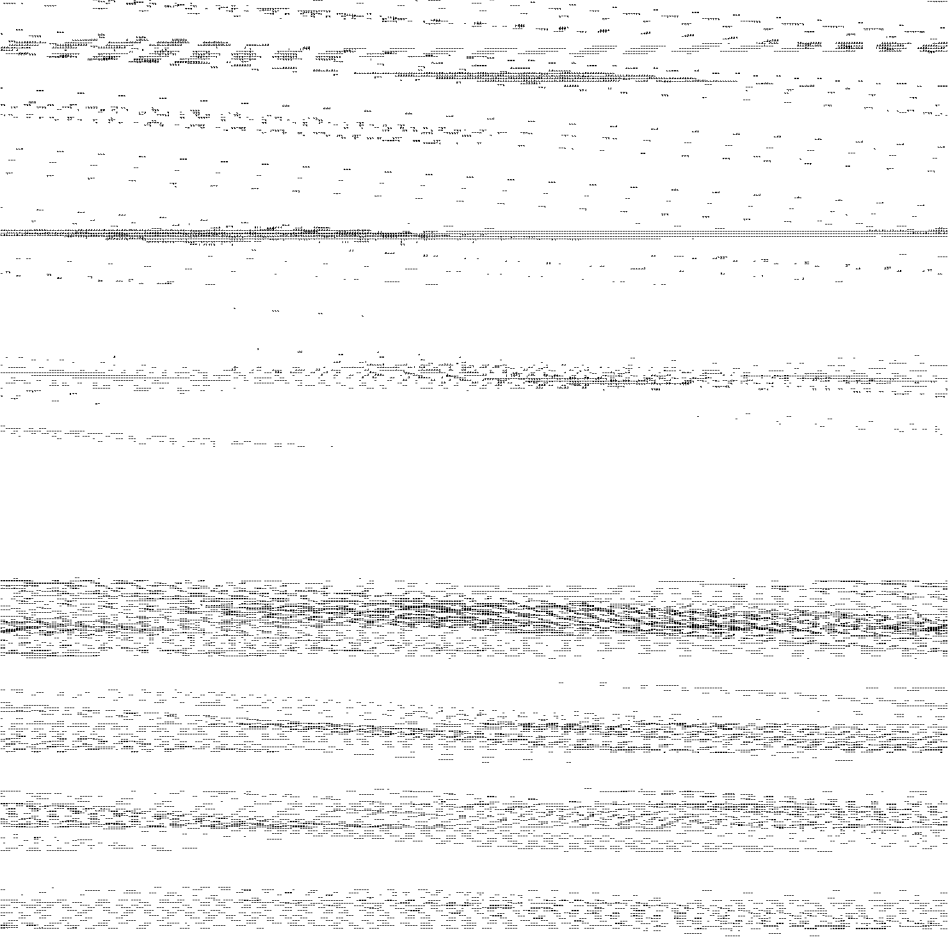
2.1 急性肝损伤组各时相血清PLD及ALT活性(见表1)

急性肝损伤时,PLD水平与ALT水平变化呈显著正相关($n=100$,$r=0.3585$,$P<0.01$)。

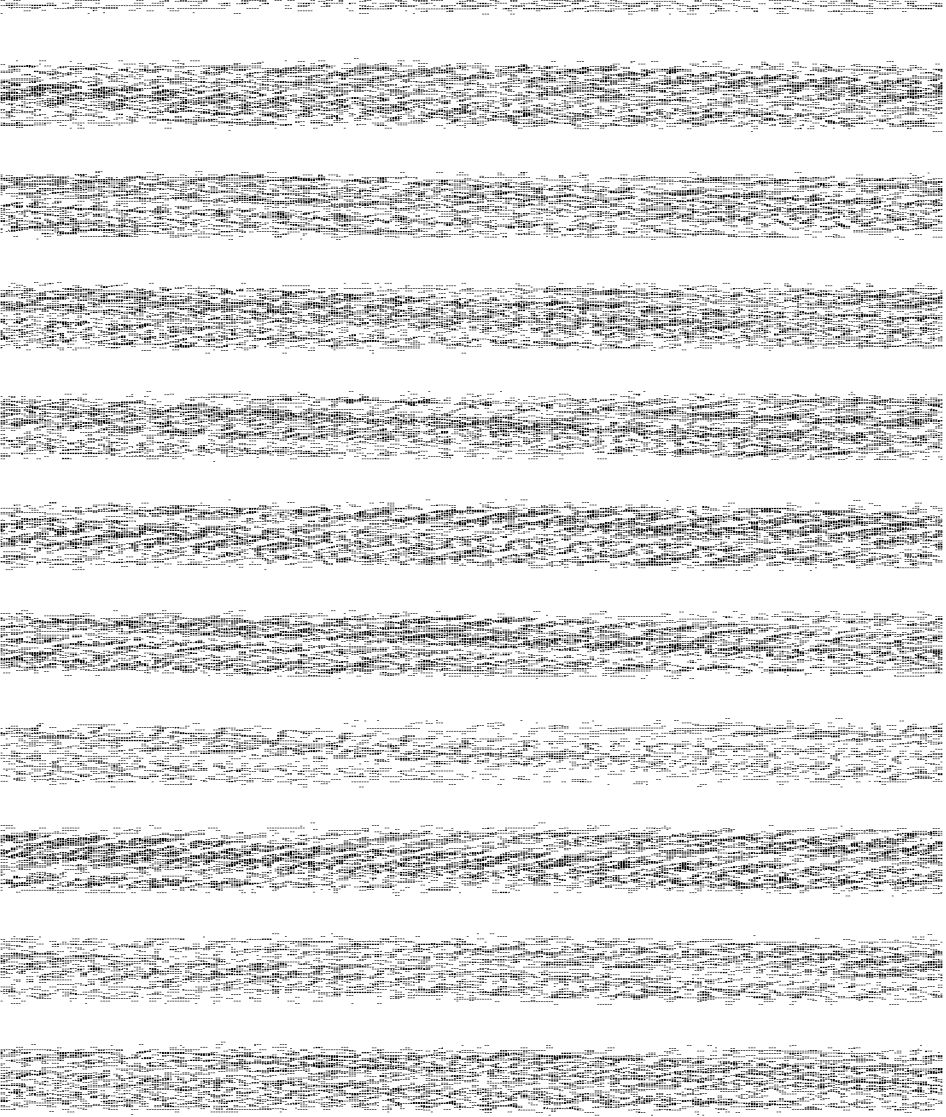
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但该项检查有一定局限性，且难以反复进行。因此，建立诊断慢性肝病的生化指标一直为临床所关注。以往的研究多集中在反映胶原蛋白合成代谢酶如单胺氧化酶，脯氨酸羧化酶[6]，以及直接测定血中前胶原Ⅰ的羧基末端肽和前胶原Ⅲ的氨基末端肽[7]、板层素[8]的研究，而直接研究反映胶原分解代谢的指标甚少。PLD是胶原降解代谢的关键酶，肝纤维化进展时，肝内胶原合成与分解代谢均增强，新生胶原纤维快速降解率为10.3%～18.%。增多的胶原降解产物氨酰基脯氨酸导致PLD活性增强[9]。

本文慢性肝损伤动物模型的连续肝组织学观察证实，小鼠硬化形成大致分为胶原增生(1～6)周肝硬化形成和肝硬化(9周以后)3个阶段。血清ALT在上述3个阶段无明显波动，呈轻度升高，为CCl_4的肝毒性所致，与胶原纤维增生和肝硬化形成无关。由于采血时间是在注射CCl_4后96 h，约2倍于PLD半寿期，PLD活性增高并非CCl_4肝毒性所致，而与胶原纤维活跃增生有关。第1周末，PLD开始升高，肝内已出现胶原纤维和成纤维细胞增生，随注射CCl_4时间延长(第3～6周)，肝内胶原纤维和成纤维细胞增生更趋活跃，PLD升至最高值，6～9周，增生的胶原纤维向肝小叶延伸，可见假小叶雏型，PLD水平呈下降趋势，9周以后，典型肝硬化形成，PLD降至正常。以上观察表明。血清PLD水平变化主要是反映胶原纤维增生和肝硬化形成过和，而与肝内胶原纤维量的多少无关。动态观察血清PLD活性变化并联合其它肝功能试验结果综合分析，将显著提高慢性肝病，尤其是早期肝硬化的诊断水平。

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The relationship of serum prolidase and liver damage in mice

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Abstract The activity of serum prolidase (PLD) and alanine aminotransferase (ALT) was determined and the histological changes of the liver was observed on a murine model of hepatocyte damage and fibrosis induced with CCl_4. In the acute hepatocyte damage, the activity of PLD and ALT was significantly increased with a positive correlation between the 2 enzymes ($n=100$, $r=0.3853$, $P<0.01$). The peak elevation of ALD was in the 16th h, corresponding to the time of most severe lysis of hepatocytes. The time of peak elevation and recovery of PLD was earlier than that of ALT. The chronic hepatocyte damage and fibrosis could roughly be divided into fibroproliferating stage (1st to 6th week), cirrhosis developing stage (6th to 9th week)and cirrhosis stage (9th to 12th week). There was no significant correlation between the changes of PLD and ALT($n=88$, $r=0.132$, $P>0.05$) and no significant changes of ALT was observed. The PLD activity began to elevate in the lst week, reached the peak in the 4th week and returned to the normal range after 9th week. These findings suggest that serum activity of both PLD and ALT is a goog biochemical index to diagnose acute hepatocyte injury. When the acute phase progresses into the chronic phase, the activity of PLD only can reveal the development of cirrhosis.

Key words prolidase; alanine aminotransferase; liver fibrosis; liver cirrhosis; mouse

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