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IL-6与整合素家族细胞黏附分子在大鼠急性坏死性胰腺炎合并多器官损伤模型中的表达

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Expression of IL-6 and integrin family cell adhesion molecules in acute necrotizing pancreatitis complicated with multiple organ injury in rats

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Abstract

AIM:To detect the expression of integrin family cellular adhesion molecules LFA-1, Mac-1 and IL-6 in acute necrotizing pancreatitis complicated with multiple organ injury.

METHODS:The flow cytometer was used to detect the expression of LFA-1 and Mac-1 on leukocyte and the radio-immunity to detect the IL-6 in acute necrotizing pancreatitis of rats at different time points.

RESULTS:The expression of LFA-1 and Mac-1 increased significantly in acute pancreatitis group compared with that in control group at all time points (P <0.01), i.e. : LFA-1: 1 h, 7.6±0.4 *vs* 22.7±1.6; 3 h, 7.9±0.5 *vs* 26.7±5.5; 6 h, 13.5±1.8 *vs* 30.3±1.6; 12 h, 9.7±0.7 *vs* 20.3±4.2; 24 h, 10.1±1.1 *vs* 15.9±0.7. Mac-1: 1 h, 6.2±1.1 *vs* 7.0±2.5; 3 h, 6.3±0.8 *vs* 36.0±1.5; 6 h, 7.9±1.2 *vs* 27.1±1.4; 12 h, 6.4±0.4 *vs* 22.5±2.1; 24 h, 7.1±0.4 *vs* 20.6±1.6. Expression of IL-6 increased significantly in acute pancreatitis group compared with that in control group, i.e.: 1 h, 65.6±3.2 *vs* 72.4±4.0 (P <0.05); 3 h, 68.2±5.5 *vs* 155.3±16.3 (P <0.01); 6 h, 69.3±2.6 *vs* 229.2±16.4 (P <0.01); 12 h, 73.4±2.6 *vs* 287.7±13.9 (P <0.01); 24 h, 76.9±3.3 *vs* 289.5±16.1 (P <0.01). Morphological examination demonstrated that inflammatory cells, insterstitial edema, interstitial hemorrhage, desquamation and disintegration occurred in the lungs and kidneys.

CONCLUSION:IL-6, LFA-1 and Mac-1 may play the very active role in acute pancreatitis.

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摘要

目的:检测IL-6与整合素家族细胞黏附分子LFA-1，Mac-1在大鼠急性坏死性胰腺炎合并多器官损伤中表达的动态变化.

方法:应用放免法连续测定大鼠急性坏死性胰腺炎合并多器官损伤模型血清IL-6的变化. 应用流式细胞仪，于多个时相点检测整合素家族细胞黏附分子LFA-1，Mac-1在SD大鼠急性坏死性胰腺炎合并多器官损伤血中性粒细胞表面表达的变化.并检测血浆淀粉酶及胰腺、肺和肾脏的病理学损害.

结果:IL-6，LFA-1和Mac-1在炎症组中各个时相点的表达均明显高于对照组，LFA-1:1 h，7.6±0.4 *vs* 22.7±1.6 (P <0.01); 3 h，7.9±0.5 *vs* 26.7±5.5 (P <0.01); 6 h，13.5±1.8 *vs* 30.3±1.66 (P <0.01); 12 h，9.7±0.7 *vs* 20.3±4.2 (P <0.01); 24 h，10.1±1.1 *vs* 15.9±0.7 (P <0.01). Mac-1:1 h，6.2±1.1 *vs* 7.0±2.5 (P <0.05); 3 h，6.3±0.8 *vs* 36.0±1.5 (P <0.01); 6 h，7.9±1.2 *vs* 27.1±1.4 (P <0.01); 12 h，6.4±0.4 *vs* 22.5±2.1 (P <0.01); 24 h，7.1±0.4 *vs* 20.6±1.6 (P <0.01). IL-6:1 h，65.6±3.2 *vs* 72.4±4.0 (P <0.05); 3 h，68.2±5.5 *vs* 155.3±16.3 (P <0.01); 6 h，69.3±2.6 *vs* 229.2±16.4 (P <0.01) ;12 h，73.4±2.6 *vs* 287.7±13.9 (P <0.01); 24 h，76.93±3.3 *vs* 289.5±16.1 (P <0.01). 炎症组中血浆淀粉酶明显高于对照组，并有明显的胰腺、肺和肾脏的病理学改变.

结论:IL-6和整合素家族细胞黏附分子LFA-1，Mac-1均参与了急性坏死性胰腺炎继发多器官功能损伤的过程.

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0 引言

急性坏死性胰腺炎(acute necrotizing pancreatitis，ANP)的发病机制至今仍未完全清楚[1-5]，研究发现过度激活的中性白细胞对自身组织的损伤是急性胰腺眼导致全

身并发症的重要原因[6-19]. 我们应用放免法和流式细胞术检测了IL-6及整合素家族细胞黏附分子LFA-1，Mac-1在ANP合并多器官功能损伤的模型中的表达，以探讨上述炎症递质在ANP合并多器官损伤中的作用及其机制.

1 材料和方法

1.1 材料 SD大鼠雌雄不拘，质量250-300 g. 随机分成对照组和ANP组，各25只. 模型制作前12 h禁食，自由饮水，吸入麻醉(氟烷、氧气、笑气)混合吸入，流量2 L/min，诱导成功后麻醉吸入量减半. ANP模型采用谷俊朝等的方法(中华实验外科杂志 1998;5:395)，上腹正中切口，长约3 cm，显露胰腺，结扎胰管于十二指肠降部胰管末端开口处，50 g/L牛磺胆酸钠10 ml/Kg于胰腺被膜下多位点注射(一般取8-10个位点)，分层关腹. 对照组仅行开腹手术，轻轻翻动十二指肠及胰腺后关腹. 牛磺胆酸钠(和光纯药工业株式会社); RaBA-Super血生化仪；Coulter Epics XL流式细胞仪；FITC标记的小鼠抗大鼠LFA-1 mAb和FITC标记的小鼠抗大鼠Mac-1 mAb均购自美国BD PharMingen公司；对照用FITC标记的兔抗羊IgG抗体及IL-6放免试剂盒购自Sigama公司.

1.2 方法 模型制作成功后分1，3，6，12和24 h五个时相点，每个时相点5只动物，麻醉下剖腹经下腔静脉取血2 mL，1 mL用20 g/L EDTA抗凝，另外1 mL行IL-6放免检测及血生化检查，测定血淀粉酶(amylase)，BUN，Cr，并取部分胰腺、肺脏、肾脏经40 g/L甲醛固定，常规石蜡切片行HE染色，观察病理改变. 抗凝血离心(1 500 r/min，10 min)；取白细胞层，经溶血素3 mL溶血15 min × 2次；离心1 500 r/min，10 min × 2次；弃上清，得白细胞加入PBS定容至1 mL；吹打沉淀，取白细胞悬液300 μl，分别置于3个流式细胞仪专用试管，每管各加100 μL；三管分别加入LFA-1 mAb、Mac-1 mAb及对照兔抗羊IgG抗体20 μL，4 ℃避光反应30 min；各管加入4 ℃ PBS洗两次，以清除未结合的抗体，800 r/min离心5 min × 2次，弃上清，加入4 ℃ PBS定容至1 mL，置于冰上至上样检测. 调整流式细胞仪的荧光检测变异系数使其稳定在2 %左右. 光源为488 nm的氩离子激光，FITC受激发后发出绿色荧光，启动Coulter流式细胞仪专用Elite分析软件，分别测定中性粒细胞前向散射光及侧向散射光，测定平均荧光密度(mean fluorescence intensity，MFI). IL-6放射免疫测定严格按照说明书步骤进行.

统计学处理 结果以均值±标准差表示，应用Excel软件行t检验.

2 结果

2.1 病理和血清学改变 炎症组各组均有血性腹水，并随模型制作后时间的延长腹腔内血性腹水量逐渐增多，胰腺周围有皂化斑形成，胰腺瘀血呈灰褐色，镜下见胰腺呈片状坏死，红细胞外渗，有大量白细胞浸润，腺胞水肿呈岛状. 肺瘀血明显，镜下见：肺间质水肿，肺泡上皮细胞肿胀有些出现坏死，并伴有白细胞浸润. 肾脏水肿明显，肾包膜呈灰白色或暗红色，镜下见肾小管上皮细胞肿胀有的出现坏死，肾间质有片状出血，中性粒细胞浸润，且肾小管内有较多管型;对照组则无明显病理改变. IL-6在ANP组各时相点表达均明显高于对照组(P <0.05或 P <0.01)，且逐渐升高. ANP组各时相点的血淀粉酶，BUN，Cr均明显高于对照组(表1).

表1 大鼠血amylase，BUN，Cr，IL-6变化($\bar{x}\pm s$)

组别	t/h				
	1 h	3 h	6 h	12 h	24 h
对照组 amylase(U/L)	70±6.5	150±11	160±17.5	167±17	171±21
ANP组 amylase(U/L)	350±12[b]	680±26[b]	840±21[b]	810±33[b]	780±17[b]
对照组 BUN(mmol/L)	4.3±0.9	5.7±1	5.5±1.3	6.1±1.5	5.9±1.5
ANP组 BUN(mmol/L)	7±1.2[a]	10.5±1.1[b]	7.1±1.5[b]	23.8±2.2[b]	24.5±1.8[b]
对照组 Cr(umol/L)	70±5.5	78±4.2	75±4.3	80±6.6	76.5±6.7
ANP组 Cr(umol/L)	77±4.9[a]	120±7.1[b]	190±5.8[b]	210±12.5[b]	225±11.7[b]
对照组 IL-6(ng/L)	65.6±3.2	68.2±5.5	69.3±2.6	73.4±2.6	76.9±3.3
ANP组 IL-6(ng/L)	72.4±4.0[a]	155.3±16.3[b]	229.2±16.3[b]	87.7±13.9*	289.5±16.1[b]

同一时相点比较 [a]P <0.05，[b]P <0.01，*vs* 对照组.

2.2 白细胞表面LFA-1，Mac-1表达 LFA-1在ANP组各时相点表达的MFI均明显高于对照组(P<0.05或 P<0.01)，LFA-1在白细胞表面表达MFI于模型制作后6 h达到最高峰. Mac-1在ANP组各时相点表达的MFI明显高于对照组(P<0.05或 P<0.01)，Mac-1在白细胞表面表达MFI于模型制作后3 h表达即达到最高峰(表2).

表2 白细胞表面LFA-1，Mac-1表达变化($\bar{x}\pm s$)

组别	t/h				
	1 h	3 h	6 h	12 h	24 h
对照组 LFA-1 MFI	7.6±0.4	7.9±0.5	13.5±1.8	9.7±0.7	10.1±1.1
ANP组 LFA-1 MFI	22.7±1.6[b]	26.7±5.5[b]	30.3±1.6[b]	20.3±4.2[b]	15.9±0.7[b]
对照组 Mac-1 MFI	6.2±1.1	6.3±0.8	7.9±1.2	6.4±0.4	7.1±0.4
ANP组 Mac-1 MFI	7.0±2.5[a]	36±1.5[b]	27.1±1.4[b]	22.5±2.1[b]	20.6±1.6[b]

同一时相点比较 [a]P <0.05，[b]P <0.01，*vs* 对照组.

3 讨论

ANP临床过程极为凶险，死亡率很高[20-23]，而早期死亡的主要原因多为器官衰竭，其发病机制仍不是很清楚，临床上也尚未发现特别有效的疗法. 目前的研究发现一些炎症递质在ANP合并多器官功能损伤时起重要作用[24-27]. 由于多种炎症递质的共同作用，导致急性胰腺炎从局部病变迅速发展为ANP，在胰腺组织大量坏死的同时，并发全身多个脏器功能障碍[28,29]. IL-6就是这些炎症递质中的一种，他促进T淋巴细胞分化增生，

促进急性期反应等作用导致组织损伤. 整合素家族细胞黏附分子中 LFA-1、Mac-1 只表达在白细胞表面，是目前被发现与炎症创伤后白细胞活化关系比较密切的整合素家族细胞黏附分子. 近年偶有其在急性胰腺炎中表达研究的报道[30-34]，但对其在胰腺炎合并多器官损伤中的动态研究至今未见报道.

我们发现，IL-6、LFA-1 和 Mac-1 在 ANP 组各时相点表达均明显高于对照组，三者的表达有明显的一致性. 同时 ANP 各组均出现胰腺、肺脏和肾脏的病理学改变和血液生化学改变. 血液生化学改变及病理学改变与 IL-6、LFA-1 和 Mac-1 上调表达具有明显的一致性. 关于 ANP 中 IL-6 与 LFA-1、Mac-1 的相互关系目前国内外尚未见报道. 我们认为 IL-6、LFA-1 和 Mac-1 均参与了 ANP 多器官损伤的过程，白细胞尤其是中性粒细胞被 ANP 发生后产生大量的前炎症因子如 IL-6 等所启动激活；导致白细胞在肺、肾、肝等脏器的脉管系统扣押；白细胞开始在上述脏器的脉管系统内皮细胞的表面黏附；黏附在内皮细胞的白细胞释放弹力蛋白酶和氧自由基等毒性物质；此类毒性物质能损伤血管的内皮细胞表面，导致脏器的微血管通透性升高，从而导致脏器的间质水肿.

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