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清肺化痰汤灌胃治疗对急性胰腺炎大鼠相关肺损伤的作用 *

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摘要 目的:探讨清肺化痰汤灌胃治疗对急性胰腺炎大鼠相关肺损伤的作用。**方法:**45 只 SD 大鼠随机分为 3 组 - 正常对照组(15 只)、模型组(15 只)、清肺化痰组(15 只),模型组与清肺化痰组都建立了急性胰腺炎大鼠相关肺损伤模型,对照组仅开腹后轻翻胰腺组织后缝合。清肺化痰组在造模前 2 h 给予清肺化痰汤 0.6 mL/100 g 灌胃,对照组与模型组给予等量生理盐水灌胃。**结果:**模型组与清肺化痰组建模后 24 h、36 h、48 h 的血清淀粉酶、IL-6、IL-8 水平都高于对照组(P<0.05),清肺化痰组低于模型组(P<0.05)。模型组与清肺化痰组建模后 24 h、36 h、48 h 的肺组织病理评分、Caspase-3 蛋白相对表达水平高于对照组(P<0.05),清肺化痰组低于模型组(P<0.05)。**结论:**清肺化痰汤灌胃治疗急性胰腺炎大鼠相关肺损伤能调节肺脏细胞凋亡水平,抑制炎症因子的释放,从而减轻肺损伤,改善大鼠的病情。

关键词:清肺化痰汤;急性胰腺炎;大鼠;肺损伤;细胞凋亡

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Effects of Qingfei Huatan Decoction on Lung Injury in Rats with Acute Pancreatitis*

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ABSTRACT Objective: To investigate the effects of Qingfei Huatan Decoction on lung injury related to acute pancreatitis in rats by intragastric administration. **Methods:** In this study, 45 SD rats were randomly divided into 3 groups-normal control group (15 rats), model group (15 rats), Qingfei Huatan group (15 rats). The model group and the Qingfei Huatan group were established lung injury related to acute pancreatitis model; the control group was only opened the abdomen and turned the pancreatic tissue slightly and sutured it. The Qingfei Huatan group was given the Qingfei Huatan Decoction 0.6 mL/100 g by gavage 2 hours before model building, and the control group and the model group were given the same amount of normal saline by gavage. **Results:** The serum amylase, IL-6, and IL-8 levels of the model group and the Qingfei Huatan group 24 h, 36 h, and 48 h after modeling were higher than those of the control group (P<0.05), and the Qingfei Huatan group was lower than the model group (P<0.05). The lung tissue pathology score and the relative expression level of Caspase-3 protein 24 h, 36 h, and 48 h after modeling of the model group and the Qingfei Huatan group were higher than those of the control group (P<0.05), and the Qingfei Huatan group was lower than the model group (P<0.05). **Conclusions:** Qingfeihuatan Decoction can regulate lung cell apoptosis and inhibit the release of inflammatory factors in the treatment of acute pancreatitis related lung injury in rats, thereby reducing lung injury and improving the condition of rats.

Key words: Qingfei Huatan Decoction; Acute Pancreatitis; Rats; Lung Injury; Apoptosis

Chinese Library Classification(CLC): R-33; R576; R285.5 Document code: A

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前言

急性胰腺炎(acute pancreatitis,AP)是临床常见的危重疾病之一,是以胰腺组织坏死、弥漫性出血为特征、累及全身多个脏器的危急重症[1,2]。该病的具体机制还不明确,可能与内质网应激、氧化应激等多种因素有关[3,4]。肺损伤是急性胰腺炎的常见的重要并发症,是该病死亡的重要原因之一,临床症状因病情的不同而异,包括呼吸困难与急性呼吸窘迫综合征等[5,6]。在急性胰腺炎的肺损伤患者中,其病理特征为肺泡Ⅱ型细胞受到破坏,破坏肺泡表面张力和肺泡的稳定性,使其对肺泡内水分的调节能力受到损伤,导致机体出现低氧血症、肺水肿、进行性呼吸困难等[7]。同时肺损伤机体可出现炎性细胞浸润和腺泡细胞的破坏,导致炎性介质释放失控,从而形成恶性循环[8,9]。清肺化痰汤由白芍、木香、元胡、柴胡、黄芩、黄连、大黄、芒硝等中药组

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成，具有通里攻下、清热燥湿解毒、活血化瘀等功效[10,11]，可多层次、多因素、多途径等方式发挥机体调节作用[12,13]。本文具体探讨了清肺化痰汤灌胃治疗对急性胰腺炎大鼠相关肺损伤的作用，以明确中药应用的价值。现总结报道如下。

1 材料与方法

1.1 主要研究材料

健康雄性清洁级 sprague-dawley(SD)大鼠 45 只购自北京联通利华生物技术有限公司(体重 200~240 g，动物合格证号：SCXKll-32-92833，纳入标准：精神活泼，体形正常，反应灵敏，毛色有光泽)，清肺化痰汤来自本院中草药房，血清淀粉酶检测试剂盒购自南京建成生物制品研究所，炎症因子酶联免疫检测试剂盒购自 BlueGene 公司，抗 Caspase-3 抗体、抗β-actin 抗体购自美国 Santa Cruz 公司，水合氯醛购自天津坦成化工技术有限公司。

1.2 动物分组与处理

将所有大鼠随机分为 3 组 - 正常对照组(15 只)、模型组(15 只)、清肺化痰组(15 只)。模型组和清肺化痰组再随机分为建模后 24 h、36 h、48 h 各时间点小组，每组 5 只大鼠。

急性胰腺炎大鼠相关肺损伤模型的建立(模型组与清肺化痰组)：动物禁食、不禁水 12 h，10 %水合氯醛 0.3 mL/100 g 腹腔麻醉，开腹后应用肠夹夹持十二指肠，阻止胃十二指肠及胆胰液流向远段十二指肠，使得十二指肠段因胃肠胰液胀满而充盈。肝门端胆管用小动脉夹夹闭，固定十二指肠，采用针头从胰胆管远端行逆行胆胰管穿刺，注入 0.1 mL/100 g 的 4 %牛磺胆酸钠 2 min 后，可见液体进入胆胰管，此时肉眼可见出血水肿、局部坏死，撤除肠夹、血管夹并关腹。对照组仅开腹后轻翻胰腺组织后缝合。

造模前 2 h 清肺化痰组给予清肺化痰汤 (0.25 g/mL)0.6 mL/100 g 灌胃，对照组与模型组给予等量生理盐水灌胃。各组大鼠术后均于背部皮下注入生理盐水 2 mL/100 g，清肺化痰组于术后给予大清肺化痰汤(0.25 g/mL)0.6 mL/100 g 灌胃，对照组与模型组给予等量生理盐水灌胃。

1.3 观察指标

(1) 大鼠麻醉后采用腹主动脉穿刺抽血 0.5~1.0 mL 左右，室温静置 2 h 后，4℃、2000 rpm 离心 10 min，分离上清血清，采用酶联免疫法检测血清 IL-6、IL-8 含量。同时采用全自动生化分析仪检测血清淀粉酶含量。(2)在无菌条件下快速摘取肺脏组织。在甲醛缓冲液中固定并进行 HE 染色，在光镜下观察肺脏的病理结构变化。肺损伤分级标准：0 级(0 分)：肺支气管、肺泡和及间质、肺血管均正常；Ⅰ级(1 分)：肺间质少量白细胞，间质及肺泡腔出血水肿范围 <25 %；Ⅱ级(2 分)：间质及部分肺泡腔有较多白细胞，间质及肺泡腔出血水肿范围 25 %~50 %；Ⅲ级(3 分)：肺泡和间质有大量白细胞聚积成团，间质及肺泡腔出血水肿范围 50 % ~75 %。(3) 取大鼠肺组织进行匀浆制备，3000 r/min 离心 15 min，取上层组织，采用 Western blot 方法检测 Caspase-3 蛋白表达水平。

1.4 统计学分析

采用 SPSS 21.00，计量资料以 $\bar{x}\pm s$ 表示(对比为 t 检验，多组间比较采用单因素方差分析(ANOVA)，计数资料用率(%)表示(对比为卡方分析)，检验水准为α=0.05。

2 结果

2.1 血清淀粉酶水平对比

模型组与清肺化痰组建模后 24 h、36 h、48 h 的血清淀粉酶水平都高于对照组(P<0.05)，清肺化痰组低于模型组(P<0.05)，见表 1。

表 1 三组建模后不同时间点的血清淀粉酶水平对比(U/L，$\bar{x}\pm s$)

Table 1 Comparison of serum amylase levels at different time points among three groups after modeling (U/L, $\bar{x}\pm s$)

Groups	n	24 h	36 h	48 h
Control group	5	1112.94± 145.01	1118.87± 184.73	1098.74± 100.74
Model group	5	5492.85± 222.98*	5502.76± 198.92*	5478.22± 204.82*
Qingfei phlegm group	5	3467.98± 189.28*#	2458.76± 201.84*#	1768.87± 108.81*#
F		39.811	45.022	52.855
P		0.000	0.000	0.000

Note: Compared with the control group, *P<0.05; compared with the model group, #P<0.05.

2.2 血清炎症因子含量对比

模型组与清肺化痰组建模后 24 h、36 h、48 h 的血清 IL-6、IL-8 含量高于对照组(P<0.05)，清肺化痰组低于模型组(P<0.05)，见表 2。

2.3 肺组织病理评分对比

模型组与清肺化痰组建模后 24 h、36 h、48 h 的肺组织病理评分高于对照组(P<0.05)，清肺化痰组低于模型组(P<0.05)，见表 3。

2.4 Caspase-3 表达对比

模型组与清肺化痰组建模后 24 h、36 h、48 h 的肺脏 Caspase-3 蛋白相对表达水平高于对照组，肺化痰组低于模型组(P<0.05)，见表 4。

3 讨论

急性胰腺炎具有病情凶险、发展迅速、常肺损伤等特点，该病的病因还不明确，不过可能是其他病变的继发表现[15]。特别是机体胰酶被不同原因的激活后，打破促炎和抗炎因子的失衡，诱发机体出现胰腺微循环障碍、肠道菌群移位，导致疾病的恶化[16,17]。急性胰腺炎在祖国医学中无专述，不过病程早期可出现气机郁滞，中期可出现气滞血瘀、湿热淤塞，晚期可导致气阴暴伤、神气失脱，在治疗上需要益气养阴、清热解毒、活血化瘀[18,19]。本研究显示模型组与清肺化痰组建模后 24 h、36 h、48 h

表 2 三组建模后不同时间点的血清炎症因子含量对比(pg/mL，$\bar{x}\pm s$)

Table 2 Comparison of serum inflammatory factor content at different time points among three groups after modeling (pg/mL, $\bar{x}\pm s$)

Groups	n	IL-6			IL-8		
		24 h	36 h	48 h	24 h	36 h	48 h
Control group	5	12.48± 1.29	11.79± 0.89	12.11± 1.48	15.09± 1.47	15.78± 2.09	15.49± 1.66
Model group	5	55.29± 1.39*	54.98± 2.19*	55.09± 1.76*	78.29± 2.00*	78.98± 1.57*	79.09± 1.77*
Qingfei phlegm group	5	32.93± 3.33*#	26.09± 1.44*#	20.87± 1.47*#	56.09± 1.44*#	36.78± 2.18*#	24.09± 1.75*#
F		12.033	14.095	15.092	19.822	21.747	23.777
P		0.000	0.000	0.000	0.000	0.000	0.000

表 3 三组建模后不同时间点的肺组织病理评分对比(分，$\bar{x}\pm s$)

Table 3 Comparison of lung tissue pathological scores at different time points among three groups after modeling (scores, $\bar{x}\pm s$)

Groups	n	24 h	36 h	48 h
Control group	5	0	0	0
Model group	5	2.73± 0.33*	2.76± 0.14*	2.77± 0.27*
Qingfei phlegm group	5	2.00± 0.32*#	1.67± 0.18*#	1.10± 0.09*#
F		9.913	8.111	7.824
P		0.001	0.005	0.008

表 4 三组建模后不同时间点的肺脏 Caspase-3 蛋白相对表达水平对比(分，$\bar{x}\pm s$)

Table 4 Comparison of relative expression levels of lung Caspase-3 protein at different time points among three groups after modeling (scores, $\bar{x}\pm s$)

Groups	n	24 h	36 h	48 h
Control group	5	0.89± 0.02	0.90± 0.03	0.89± 0.02
Model group	5	5.02± 0.18*	5.18± 0.22*	5.08± 0.33*
Qingfei phlegm group	5	3.11± 0.16*#	2.00± 0.18*#	1.45± 0.14*#
F		12.093	16.035	21.774
P		0.000	0.000	0.000

的血清淀粉酶水平、肺组织病理评分都高于对照组，清肺化痰组低于模型组，表明清肺化痰汤灌胃治疗急性胰腺炎大鼠能减轻肺损伤，降低血清淀粉酶水平。从机制上分析，清肺化痰汤能改善肠蠕动功能，减轻肺组织因缺血导致的损伤，保护肠粘膜屏障，减少肠道菌群移位，减轻肺水肿[20,21]。并且该药可改善胰腺腺泡细胞损伤和器官坏死程度，从而达到减轻胰腺组织损伤的作用[22,23]。

急性胰腺炎的发病开始是由于各种始动因素破坏胰腺的正常保护机制，是机体出现多脏器功能衰竭和死亡的原因[24]。特别是当急性胰腺炎发生时，在损伤因子作用下，单核细胞被激活并释放多种细胞因子，促炎炎症反应失控，导致机体出现全身炎症反应[25]。炎症细胞因子 IL-6、IL-8 水平与急性胰腺炎病情的严重程度密切相关，它们的释放失控，可使机体释放抗炎细胞因子减少，从而导致患者的病情恶化[26]。本研究显示模型组与清肺化痰组建模后 24 h、36 h、48 h 的血清 IL-6、IL-8 含量高于对照组，清肺化痰组低于模型组，表明清肺化痰汤灌胃治疗急性胰腺炎大鼠能抑制炎症因子的释放。与于家川[27]等学者的研究类似，该学者探讨清胰汤联合骨髓间充质干细胞在治疗急性胰腺炎相关性肺损伤时的保护机制，结果显示模型组的 IL-6、IL-10 均高于对照组，清胰汤治疗组低于对照组，说明清胰汤通过防止过氧化损伤，纠正机体致炎与抗炎系统失衡对肺脏起保护作用。本研究采用的清肺化痰汤从机制上分析，白芍、木香、元胡可清热燥湿、泻火解毒、攻积导滞，达到减轻肺及肠道损伤的作用；柴胡、黄芩、黄连、大黄、芒硝等可肝解郁、行气消胀、利胆护肝，从而达到减轻全身炎症反应及控制感染的作用[28,29]。

肺脏组织细胞凋亡可保护肺脏损伤，并可减轻急性胰腺炎的病情[30,31]。细胞内存在多条细胞凋亡信号通路，特别是活化的 Caspases-3 可经蛋白酶解过程激活后，从而导致细胞凋亡指数增加，Caspases-3 表达影响缺失可加重急性胰腺炎的病情[32,33]。本研究显示模型组与清肺化痰组建模后 24 h、36 h、48 h 的肺脏 Caspase-3 蛋白相对表达水平高于对照组，肺化痰组低于模型组，表明清肺化痰汤灌胃治疗急性胰腺炎大鼠能调节 Caspase-3 蛋白表达水平，从而影响肺脏组织细胞的凋亡。

总之，清肺化痰汤灌胃治疗急性胰腺炎大鼠相关肺损伤能调节肺脏细胞凋亡水平，抑制炎症因子的释放，从而减轻肺损伤，改善大鼠的病情。本研究也存在一定的不足，没有对肺损伤组织病理进行电镜观察，且机制分析还不够深入，同时没有设立其他药物对照组，对结果不能更好的进行说明，将在后续研究中进行探讨。

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