

· 论 著 · doi:10.3969/j.issn.1671-8348.2016.26.003

芥子气经腹腔和气管致大鼠急性肺损伤炎症反应的比较研究*

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[摘要] **目的** 经腹腔和气管建立大鼠芥子气(SM)肺损伤的动物模型,比较两种大鼠急性肺损伤模型炎症反应的差异。**方法** 选取 Sprague Dawley 大鼠 136 只,分为 5 组,正常对照组 8 只,其他 4 个组(腹腔 SM 组、腹腔丙二醇对照组、气管 SM 组、气管丙二醇对照组)每组 32 只。腹腔 SM 组腹腔内注入稀释的 SM 0.1 mL(0.96 LD₅₀ = 8 mg/kg),气管 SM 组气管内注入稀释的 SM 0.1 mL(0.98 LD₅₀ = 2 mg/kg),正常对照组不做任何处理。ELISA 法检测支气管肺泡灌洗液和血液标本,HE 染色和免疫组织化学判断炎症反应情况。**结果** 腹腔 SM 组各时间段支气管肺泡灌洗液蛋白含量和细胞计数与气管 SM 组相比显著升高($P < 0.05$);腹腔 SM 组各时间段血清 TNF- α 、IL-1 β 、IL-6 与气管 SM 组相比显著升高($P < 0.05$);腹腔 SM 组各时间段肺泡间隔 T、B 淋巴细胞和巨噬细胞阳性表达率与气管 SM 组相比显著增加($P < 0.05$)。**结论** 大鼠在 SM LD₅₀ 相似的情况下,腹腔 SM 组支气管肺泡灌洗液、肺泡间隔及血清炎症反应指标明显高于气管 SM 组。

[关键词] 芥子气;肺/损伤;炎症反应;大鼠

[中图分类号] R114

[文献标识码] A

[文章编号] 1671-8348(2016)26-3608-03

A comparative study on inflammatory response due to sulfur mustard-induced acute lung injury in rat via the intraperitoneal and tracheal injection*

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[Abstract] **Objective** The purpose of this study was to establish animal model of sulfur mustard (SM)-induced acute lung injury in rats via the intraperitoneal and the tracheal injection, in order to compare the difference of inflammatory reaction. **Methods** 136 male Sprague Dawley rats were selected, then were randomly divided into the five groups, the control group with 8 cases, other four groups (i. e. the intraperitoneal SM group, the intraperitoneal propylene glycol group, the tracheal SM group, the tracheal propylene glycol group) with 32 cases in each group. The intraperitoneal SM group were injected intraperitoneally with diluted SM 0.1 mL(0.96 LD₅₀ = 8 mg/kg), the tracheal SM group were injected intratracheally with diluted SM 0.1 mL(0.98 LD₅₀ = 2 mg/kg), meanwhile the status quo was kept with the normal group. SM-induced inflammatory reaction was observed by bronchoalveolar lavage fluid (BALF), serum examination, Hematoxylin Eosin staining, and immunohistochemical staining. **Results** Compared with the tracheal SM group at different time, protein contents and cell counts of BALF in the intraperitoneal SM group were significantly increased, respectively ($P < 0.05$). Compared with the tracheal SM group at different time, the levels of serum TNF- α , IL-1 β , IL-6 in the intraperitoneal SM group were significantly increased, respectively ($P < 0.05$). The positive expression ratio of T lymphocytes, B lymphocytes and macrophages in intraperitoneal SM group at different time were increased compared with the tracheal SM group, respectively ($P < 0.05$). **Conclusion** Under similar SM LD₅₀ in rat, in the intraperitoneal SM group, inflammatory reaction of BALF, alveolar septum, and serum were significantly higher than in the tracheal SM group.

[Key words] mustard gas; lung/injuries; inflammatory reaction; rat

芥子气(Sulfur mustard, SM)是一种亲脂性烷化剂,可迅速穿透上皮组织导致皮肤或呼吸道损伤^[1-2]。皮肤、眼睛和呼吸道是 SM 攻击的主要靶器官,其损伤程度与剂量和持续时间密切相关^[3]。SM 肺损伤早期死亡原因为肺部感染和呼吸衰竭^[4]。SM 可触发促炎反应通路,炎症因子介导炎性细胞肺浸润,并贯穿于肺损伤的全过程^[5-6]。SM 经皮肤、皮下、口服使小鼠染毒,以经皮肤致肺损伤的组织学改变最明显^[7]。有关 SM 肺损伤炎症反应的实验指标,国内文献报道甚少。本文通过建立经腹腔和气管 SM 肺损伤大鼠模型,比较支气管肺泡灌

洗液和血清及肺泡间隔的炎症反应指标,旨在评估 2 种途径 SM 肺损伤的差异性。

1 材料与方法

1.1 材料 所有动物经潍坊医学院动物伦理委员会批准。选取健康雄性 Sprague Dawley 大鼠(SPF 级,中国人民解放军军事医学科学院实验动物中心,合格证号:0015902)136 只,体重 280~300 g,年龄 15 周。将大鼠分为腹腔 SM 组(32 只)、腹腔丙二醇组(32 只)、气管 SM 组(32 只)、气管丙二醇组(32 只)、正常对照组(8 只)。

1.2 方法

1.2.1 动物模式的建立 SM液(纯度>90%)临用前用丙二醇稀释至所需浓度。(1)气管途径染毒动物模型建立:实验前气管SM组和气管丙二醇组皮下注射阿托品(0.05 mg/kg),30 min后腹腔内注射盐酸氯胺酮(100 mg/kg)实施麻醉,气管内注入稀释的SM 0.1 mL(0.98 LD₅₀ = 2 mg/kg),气管丙二醇组注入丙二醇 0.1 mL。(2)腹腔途径染毒动物模型建立:同上方法实施麻醉。腹腔SM组大鼠腹腔内注入稀释的SM 0.1 mL(0.96 LD₅₀ = 8 mg/kg),腹腔丙二醇组注入丙二醇 0.1 mL,正常对照组不做任何处理。1,2-丙二醇溶液由天津致远化学有限公司提供。

1.2.2 支气管肺泡灌洗液测定 腹腔和气管SM组大鼠,在染毒6、24、48、72 h后,腹腔注射3%戊巴比妥(30 mg/kg),麻醉后打开胸腔,心脏抽血2 mL,放血处死,然后结扎右侧肺门。气管做“T”形切口,静脉导管(外径1.8 mm)插入左主支气管。抽取预热(37.3~37.5 °C)生理盐水2.5 mL,缓慢注入,然后回抽灌洗液。反复抽注10次,每只大鼠灌洗5次,抽液注入离心管内(冰浴)。标本4 °C离心(223.6×g离心10 min),上清液肝素抗凝,-80 °C保存备用。采用全自动生化免疫一体机(COBAS 8000型,德国罗氏公司)进行蛋白含量测定。1 mL磷酸盐缓冲液(PBS)再悬浮细胞沉淀,用台盼蓝染色,取10 μL加入细胞计数板,光镜(BX51型,日本奥林巴斯公司)下细胞计数。

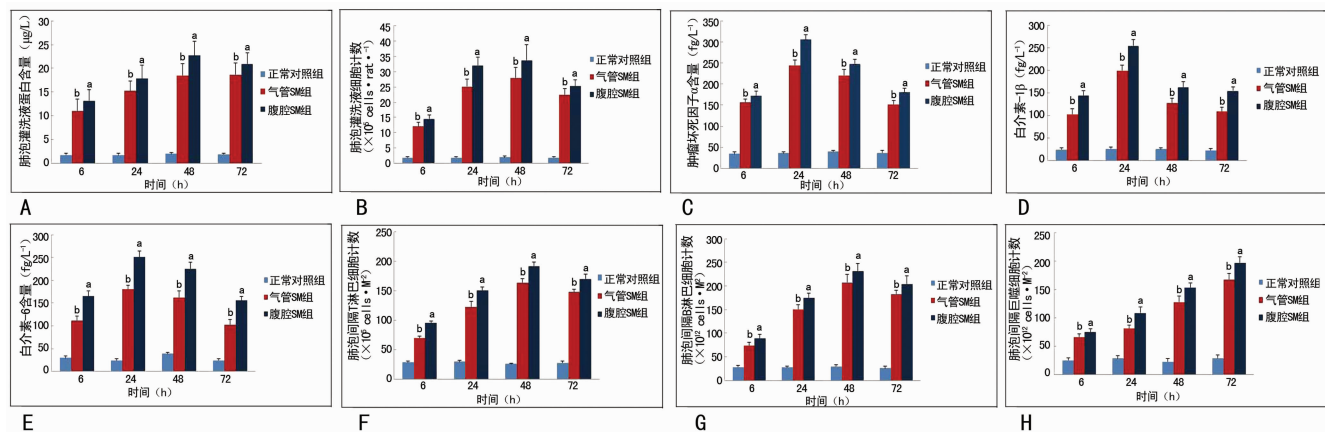
1.2.3 血清炎症因子测定 将腹腔SM组和气管SM组不同时间段获取的大鼠血2 mL,37 °C水浴1 h,4 °C过夜,然后223.6×g离心10 min,取上清液,分装于无菌小瓶中,-80 °C保存备用。采用酶标仪(Versa Max型,美国Molecular Devices公司),检测血清肿瘤坏死因子α(TNF-α)、白细胞介素(IL)-1β、IL-6浓度。ELISA试剂盒由深圳科润达生物工程有限公司提供,所有流程严格按照说明书进行操作。

1.2.4 免疫组织化学 每一个标本切取15份,每5份一组进行免疫组化染色。pH 8.5,乙二胺四乙酸(EDTA)抗原修复,0.3% H₂O₂和山羊血清封闭,免疫组织化学采用SP法,一抗4 °C孵育过夜(兔抗大鼠CD4单克隆抗体标记T淋巴细胞,兔抗大鼠CD20单克隆抗体标记B淋巴细胞,兔抗大鼠CD68单克隆抗体标记巨噬细胞),DAB显色,苏木素复染,封片。阴性对照以PBS代替一抗。CD4、CD20、CD68试剂盒由北京中杉金桥生物技术有限公司提供。

1.3 统计学处理 采用SPSS17.0统计软件,计量资料用 $\bar{x} \pm s$ 表示,组间比较采用两独立样本t检验,以P<0.05为差异有统计学意义。

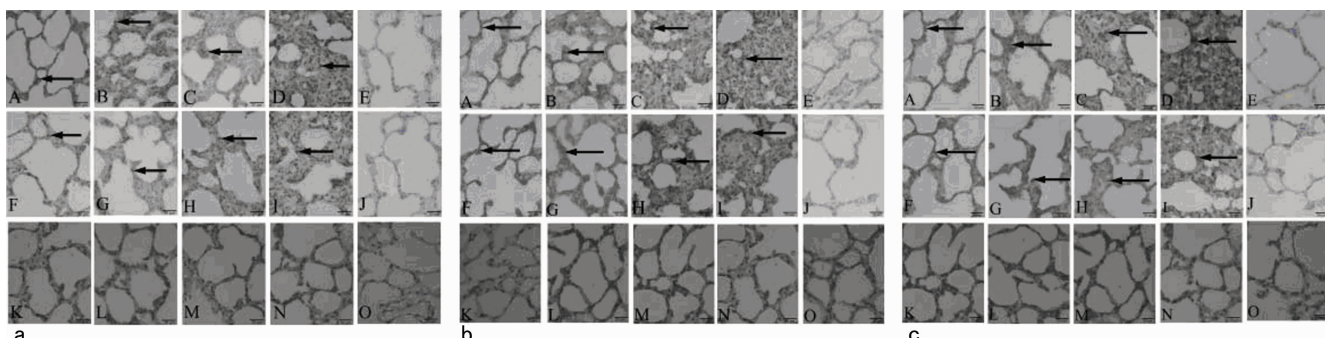
2 结果

2.1 支气管肺泡灌洗液蛋白和细胞分析 腹腔和气管SM组支气管肺泡灌洗液中蛋白含量和细胞计数均48 h达高峰。腹腔SM组各时间段蛋白含量和细胞计数与气管SM组相比明显升高(图1A、B)。



A:支气管肺泡灌洗液蛋白含量;B:支气管肺泡灌洗液细胞计数;C:血清TNF-α水平;D:血清IL-1β水平;E:血清IL-6水平;F:肺泡间隔CD4阳性表达率;G:肺泡间隔CD20阳性表达率;H:肺泡间隔CD68阳性表达率。^a:P<0.05,与气管SM组比较;^b:P<0.05,与正常对照组比较。

图1 大鼠支气管肺泡灌洗液和血清及肺泡间隔炎症反应变化趋势



a:CD4表达;b:CD20表达;c:CD68表达。A~D:6、24、48、72 h腹腔SM组阳性表达;E:正常对照组(箭头示阳性表达,标尺为20 μm)。F~I:6、24、48、72 h气管SM组阳性表达;J:正常对照组(箭头示阳性表达,标尺为20 μm)。K~N:6、24、48、72 h气管丙二醇对照组;O:正常对照组(标尺为20 μm)。

图2 大鼠肺泡间隔T淋巴细胞、B淋巴细胞、巨噬细胞表达(×400)

2.2 血清炎性因子分析 腹腔和气管 SM 组血清 TNF- α 、IL-1 β 、IL-6 水平 24 h 达高峰,腹腔 SM 组各时间段血清炎性因子水平与气管 SM 组相比明显升高(图 1C~E)。

2.3 大鼠肺泡间隔炎细胞浸润

2.3.1 腹腔 SM 组(CD4)6、24、48 h 肺泡间隔 T 淋巴细胞聚集成簇,72 h 呈团簇状。气管 SM 组(CD4)6、24、48、72 h 肺泡间隔 T 淋巴细胞聚集成簇。丙二醇和正常对照组(CD4)呈零星分布(图 2a A~O)。腹腔 SM 组各时间段肺泡间隔 T 淋巴细胞阳性表达率与气管 SM 组相比明显增多(图 1F)。

2.3.2 腹腔和气管 SM 组(CD20)6 h 肺泡间隔 B 淋巴细胞呈带状分布,24、48、72 h 聚集成簇。丙二醇和正常对照组呈零星分布(图 2b A~O)。腹腔 SM 组各时间段肺泡间隔 B 淋巴细胞阳性表达率与气管 SM 组相比明显增多(图 1G)。

2.3.3 腹腔和气管 SM 组(CD68)6 h 肺泡间隔巨噬细胞呈散在分布,24 h 增多,48、72 h 明显增多。丙二醇和正常对照组呈零星分布(图 2c A~O)。腹腔 SM 组各时间段肺泡间隔巨噬细胞阳性表达率与气管 SM 组相比明显增多(图 1H)。

3 讨 论

SM 诱导肺损伤涉及炎性介质和炎性细胞反应。Mcclintock 等^[8]研究发现,大鼠气管内滴注 2-氯乙基乙基硫醚(CEES)6 mg/kg,24 h 肺泡内可发生出血、水肿、巨噬细胞和单核细胞聚集。另有学者发现,CEES 可诱导促炎因子 IL-6 和 IL-1 β 上调,同时转录因子血清加速因子-1 (serum accelerator factor-1,SAF-1)/癌基因相关锌指蛋白 (myc-associated zinc finger protein,MAZ) 活性增加^[9]。豚鼠 CEES 染毒后检测血清发现,24 h 血清 TNF- α 、IL-1 β 、IL-6、IL-8 水平升高^[10]。可见,在 SM 诱导机体应激状态下,炎性细胞能释放促炎介质和细胞因子,刺激中性粒细胞的溢出和集聚^[11-13]。在损伤部位,中性粒细胞也可通过脱颗粒和髓过氧化物酶的释放来改变组织的微环境^[14]。

本研究发现,腹腔和气管 SM 组炎性反应指标的变化具有如下特点:(1)支气管肺泡灌洗液蛋白含量和细胞计数 48 h 达高峰;(2)血清促炎因子 TNF- α 、IL-1 β 、IL-6 水平 24 h 达高峰;(3)免疫组织化学显示肺泡间隔 T、B 淋巴细胞和巨噬细胞浸润随时间延长增多;(4)上述炎性反应指标腹腔 SM 组与气管 SM 组相比明显升高。本研究支气管肺泡灌洗液中蛋白含量和细胞计数与 Anderson 等^[15]和 Calvet 等^[16]报道一致。促炎因子水平与 Yego 等^[10]和 Emad 等^[17]报道一致,与 Yaraee 等^[18]和 Pourfarzam 等^[19]报道相反。笔者认为,支气管肺泡灌洗液蛋白含量和细胞计数增多,可能与肺间质毛细血管和肺上皮细胞通透性增加有关,属一种肺实质伴随肺结构改变的炎性反应。本研究还发现,SM 致急性肺损伤炎细胞浸润以淋巴细胞为主,这与文献报道以中性粒细胞和巨噬细胞浸润为主不一致^[20]。分析可能与 SM 诱导细胞死亡,促炎介质(TNF- α 、IL-6、IL-1 β 、IL-8 等)释放到细胞外基质中,激活巨噬细胞和肥大细胞,启动免疫反应有关。与此同时,炎性细胞能释放促炎介质和化学引物,在损伤部位刺激中性粒细胞溢出与集聚^[13,21]。本研究还显示,两种途径和浓度 SM 致急性肺损伤动物模型,肺泡间隔有大量淋巴细胞浸润,中量巨噬细胞浸润,其肺损伤程度与时间和细胞密度相关。这表明 SM 诱导急性肺损伤免疫反应和炎性反应共存,以免疫反应为主导。文献^[22]报道,SM 腹腔注射引起的肺损伤比经皮下注射或口服途径更严重。当大鼠经腹腔注射 SM 剂量高于 10 mg/kg 时,就会出现大鼠死亡^[23]。有学者发现,大鼠 SM 气管内吸入剂量(1.4 mg/kg),可产生明显的肺脏炎性反应^[24]。所以,在预期实验设计

的基础上选择 SM 剂量(0.96 LD₅₀ = 8 mg/kg)腹腔造模和(0.98 LD₅₀ = 2 mg/kg)气管造模。本研究提示,大鼠在 SM LD₅₀ 相似的情况下,SM 经腹腔染毒肺炎性反应指标比经气管明显升高。分析大鼠腹膜的腹膜对 SM 的接触和吸收远远大于气管的黏膜,由此存在毒素吸收入血的浓度差异,且 SM 的剂量与组织和血的炎性反应程度呈正相关。SM 腹腔染毒致大鼠急性肺损伤炎性反应重,推测可能与腹膜对 SM 的快速吸收,血中 SM 的浓度迅速升高有关。在未来的战争和恐怖事件中,很难预测 SM 的染毒方式和剂量。本研究阐述的 SM 相关机制与获得的参数,可为 SM 的预防与治疗提供借鉴。

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- (收稿日期: 2016-03-05 修回日期: 2016-05-26)
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- (收稿日期: 2016-03-18 修回日期: 2016-06-01)