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甘草酸能减轻小鼠肺炎病毒引起的小鼠肺脏损伤

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[摘要] **目的** 通过小鼠肺炎病毒(pneumonia virus of mice, PVM)感染近交系BALB/c小鼠建立病毒感染肺炎动物模型, 观察PVM感染过程中促炎症警报素分子高迁移率族蛋白B1(high mobility group box 1, HMGB1)的变化, 以及HMGB1抑制剂甘草酸(glycyrrhizic acid, GA)对小鼠肺脏感染损伤的在体干预作用。**方法** 将3周龄的雌性BALB/c小鼠随机分为3组, 每组6只。一组未经PVM感染, 作为对照组(Control); 另外两组先以 1×10^4 半数组织培养感染剂量(50% tissue culture infective dose, TCID₅₀)/25 μ L剂量滴鼻接种PVM, 然后分别给予GA生理盐水溶液灌胃(GA组)或单纯生理盐水灌胃(normal saline, NS组)处理, 连续15 d。其间, 观察并记录各组小鼠体重、外观等改变。在实验终点采集各组小鼠的肺脏组织样本, 通过苏木精-伊红染色和免疫组织化学法检测小鼠肺脏组织内PVM和HMGB1蛋白的分布情况; 通过实时荧光定量PCR法检测小鼠肺脏组织中HMGB1、Toll样受体4(Toll-like receptor 4, TLR-4)、晚期糖基化终产物特异性受体(advanced glycosylation end-product-specific receptor, AGER), 以及白细胞介素(interleukin, IL)-1 β 、IL-2和肿瘤坏死因子 α (tumor necrosis factor- α , TNF- α)等炎症因子的表达水平。**结果** 与Control组相比, NS组小鼠6 d后体重显著下降($P < 0.05$), 组织病理学结果显示其肺脏有明显的炎症病变, 免疫组织化学结果显示HMGB1从细胞核释放至细胞质, 实时荧光定量PCR结果显示HMGB1、IL-1 β 和IL-2的表达水平均显著上调($P < 0.05$); GA干预组的临床症状和体重无明显变化。而与NS组相比, GA干预组肺组织的病理损伤明显减轻, 且肺组织中HMGB1、IL-1 β 、IL-2和干扰素 γ (interferon- γ , IFN- γ)的表达水平显著下降($P < 0.05$), 但AGER的表达水平显著增高($P < 0.05$)。**结论** PVM感染能引起明显的小鼠肺部炎症性病理损伤, 而GA能有效减轻其损伤, 其作用机制可能与激活HMGB1信号通路相关。

[关键词] 甘草酸; 高迁移率族蛋白B1; 小鼠肺炎病毒; 肺损伤; BALB/c小鼠

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Glycyrrhizic Acid Showed Therapeutic Effects on Severe Pulmonary Damages in Mice Induced by Pneumonia Virus of Mice Infection

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[ABSTRACT] Objective In this study, inbred BALB/c mice infected with the pneumonia virus of mice (PVM) were used to establish an animal model of viral pneumonia, and the changes in the pro-inflammatory alarmin molecule, high mobility group box 1 protein (HMGB1), during PVM infection were observed, as well as the *in vivo* intervention effects of the HMGB1 inhibitor, glycyrrhizic acid (GA), on PVM-induced lung injury. **Methods** Three-week-old female BALB/c mice were randomly divided into three groups, each consisting of 6 mice. One group, uninfected by PVM, served as the control group (Control). The other two groups were inoculated intranasally with PVM at a dose of 1×10^4 50% tissue culture infective dose (TCID₅₀)/25 μ L, and subsequently treated with GA saline solution (GA group) or plain saline solution (normal saline, NS group) via gavage for 15 consecutive days. During this period, changes in body weight and appearance were monitored in each group. At the end of the experiment, lung tissue samples were collected from all groups. The distribution of PVM and HMGB1 proteins in the lung tissues was analyzed using hematoxylin-eosin staining and immunohistochemistry. The expression levels of HMGB1 and its Toll-like receptor 4 (TLR-4), advanced glycosylation end-product-specific receptor (AGER), and inflammatory cytokines such as interleukin (IL)-1 β , IL-2, and tumor necrosis factor- α (TNF- α) in lung tissues of mice were measured using real time fluorescence quantitative PCR. **Results** Compared with the Control group, the NS group showed a significant weight loss after 6 days ($P < 0.05$). Histopathological tests revealed pronounced inflammatory lesions in their lungs. Immunohistochemistry results showed that HMGB1 was released from the nucleus to the cytoplasm, and real time fluorescence quantitative PCR results indicated that the expression levels of HMGB1, IL-1 β , and IL-2 were significantly upregulated ($P < 0.05$). In the GA group, there was no significant change in the clinical symptoms or body weight. However, compared with the NS group, the pathological damages of lung tissues in the GA group were significantly reduced, and the expression levels of HMGB1, IL-1 β , IL-2, and interferon- γ (IFN- γ) in lung tissues were also significantly decreased ($P < 0.05$), although the expression level of AGER was significantly increased ($P < 0.05$). **Conclusion** PVM infection can cause significant inflammatory pathological lung damages in mice, and GA can effectively alleviate the damages. Its therapeutic effect may be related to the activation of HMGB1 signaling pathway.

[Key words] Glycyrrhizic acid; High mobility group box 1; Pneumonia virus of mice; Lung damage; BALB/c mice

流感病毒、冠状病毒等是常见的呼吸道易感病毒，感染后可导致严重的肺部损伤。由病毒感染引起的免疫反应可以抑制病原体的增殖，而免疫反应的同时也可能造成严重的病理损害。已有研究发现，病毒感染可促进支气管肺泡灌洗液^[1-2]中高迁移率族蛋白B1 (high mobility group box 1, HMGB1) 及其他细胞因子的分泌，而HMGB1能诱导炎症反应，并通过旁分泌机制促进感染过程中的组织损伤^[3]。小鼠肺炎病毒 (pneumonia virus of mice, PVM) 作为一种包膜单链RNA病毒，是啮齿类动物体内的一种自然病原体^[4-5]，感染后能够产生类似人类呼吸道合胞病毒 (respiratory syncytial virus, RSV) 病原体感染的临床和病理特征，可用于探索急性呼吸道病毒感染机制^[6-7]。

HMGB1参与染色质重塑，从损伤细胞中释放，被视为一种促炎症警报素^[8]。巨噬细胞等一些炎症细胞可主动分泌HMGB1^[9]。HMGB1作为一种损伤相关分

子模式 (damage-associated molecular pattern, DAMP) 分子，可与晚期糖基化终产物特异性受体 (advanced glycosylation end-product-specific receptor, AGER) 和Toll样受体4 (Toll-like receptor 4, TLR-4) 等相应受体结合^[10]，从而调节免疫和炎症反应。因此，HMGB1被认为是一种炎症介质。

甘草是一种广泛使用的传统中草药。从甘草根中提取的甘草酸 (glycyrrhizic acid, GA)^[11]可以抑制病毒对易感细胞的吸附和渗透，并通过减少活性氧的产生来抑制病毒复制过程中所需信号因子如核因子 κ B (nuclear factor kappa B, NF- κ B)、c-Jun氨基末端激酶 (c-Jun N-terminal kinase, c-JNK)、p38的激活。GA还可以诱导内源性干扰素的产生，进而抑制病毒的复制。此外，GA可与HMGB1 DNA结合位点结合，还可逆转细胞外HMGB1的释放，抑制caspase-1的激活，避免肺组织细胞死亡^[12]，是一种有效的HMGB1拮抗

剂。GA对小鼠流感病毒感染^[13]和小鼠肺腺癌^[13]有保护作用。为此,本研究用PVM感染近交系BALB/c小鼠建立病毒感染肺炎动物模型,观察GA对肺损伤及肺组织中HMGB1信号通路相关分子表达水平的影响,以期为病毒感染所致肺部损伤的病理机制及其干预技术的研究提供基础。

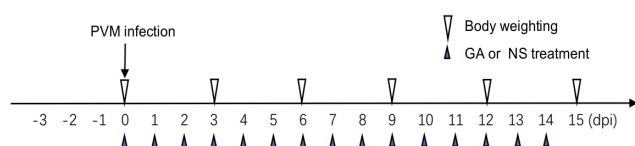
1 材料与方法

1.1 病毒制备

PVM (VR-25) 来自美国模式菌种保藏中心 (American Type Culture Collection, ATCC), 由本实验室保存, 使用时采用半数组织细胞感染剂量 (50% tissue culture infective dose, TCID₅₀) 进行定量。

1.2 动物实验

SPF级3周龄雌性BALB/c小鼠购自北京华阜康生物科技股份有限公司 [SCXK (京) 2014-0004, 质量合格证号为No.11401300088573、11401300088574], 饲养在动物生物安全二级 (animal bio-safety level-2, ABSL-2) 设施 [SYXK (京) 2015-0035] 的独立通风笼 (individually ventilated cage, IVC) 中。小鼠随机分为3组, 每组各6只: 对照组 (Control) 小鼠不感染PVM; 感染组 (NS组) 小鼠给予PVM感染, 同时给予0.9%NaCl溶液 (即生理盐水) 处理; GA治疗组 (GA组) 小鼠在感染病毒的同时给予GA (MedChemExpress, CAT: HY-N0184) 治疗。动物实验于2019年2月开展, 流程时间表见图1。



注: 在小鼠肺炎病毒 (PVM) 感染前3 d, 将所有小鼠引入动物生物安全二级 (ABSL-2) 设施。第0天, 测量小鼠体重, 进行PVM滴鼻接种, 同时分别灌胃给药, 甘草酸 (GA) 或生理盐水 (NS)。此后, 每日给予GA或NS治疗, 每3 d称重一次。每组6只小鼠。实验终点设为病毒感染后第15天。

Note: Three days before pneumonia virus of mice (PVM) infection, all mice were transferred to the ABSL-2 facility. On Day 0, the body weights of mice were measured, PVM intranasal inoculation was performed, and glycyrrhizic acid (GA) or normal saline (NS) was administered via gavage. Subsequently, GA or NS treatment was given daily and body weights were measured every 3 days. Each group consisted of 6 mice. The endpoint of the experiment was set at 15 dpi (day post infection).

图1 动物实验时间表

Figure 1 Animal experiment timeline

腹腔注射200 mg/kg 苯巴比妥钠 (Sigma-Aldrich, CAS: 57-30-7), 使小鼠处于轻度麻醉状态下, 以 1×10^4 TCID₅₀/25 μ L 剂量滴鼻接种PVM。NS组小鼠灌胃生理盐水。GA组小鼠灌胃GA生理盐水溶液 (GA以5 mg/mL 质量浓度溶解于生理盐水, 按小鼠体重以50 mg/kg 吸取相应量GA溶液, 并补入生理盐水, 使每只小鼠每天总灌胃量为200 μ L)。连续灌胃15 d, 并定期记录动物体重和外观等改变。在实验终点脱颈椎处死动物, 采集组织样本, 即从单个小鼠身上统一收集左肺一片肺叶, 并在液氮中快速冷冻。其他肺组织用中性甲醛溶液固定, 进行组织病理学检测。所有动物实验操作均符合实验动物伦理和福利要求, 并经中国医学科学院医学实验动物研究所实验动物使用与管理委员会批准 (XZG18001)。

1.3 苏木精-伊红染色和免疫组织化学分析

经中性甲醛溶液固定的肺组织在梯度浓度的乙醇溶液中脱水后包埋于石蜡中, 以4 μ m 厚度切片, 进行苏木精-伊红染色。通过免疫组织化学分析采用本实验室自行制备的大鼠抗PVM血清作为一抗检测组织切片中的PVM。大鼠抗PVM血清制备过程: 以 1×10^4 TCID₅₀/25 μ L PVM 滴鼻接种SD大鼠, 间隔2周反复接种3次, 收集动物外周血后, 以12 000 r/min 离心收集血清, 然后在56 $^{\circ}$ C下灭活, 在测试抗血清滴度后, 将其分装冻存于-80 $^{\circ}$ C的环境中。以大鼠正常血清为阴性对照, 采用购自Thermo Fisher公司的兔抗HMGB1抗体 (PA5-79373) 作为一抗, 通过免疫组织化学法检测组织切片中的HMGB1蛋白; 二抗选用辣根过氧化物酶标记的山羊抗大鼠IgG多克隆抗体; 3,3',5,5'-四甲基联苯胺 (3,3',5,5'-tetramethyl benzidine, TMB) 显色, 苏木精对比染色细胞核。

1.4 实时荧光定量PCR检测

冷冻的小鼠肺组织样品在TRIzol 裂解缓冲液 (Invitrogen, Carlsbad, California) 中研磨, 提取组织总RNA。使用PrimeScript RT试剂盒 (TaKaRa, 大连) 进行逆转录以制备组织cDNA, 后使用SYBR RT-PCR试剂盒 (TaKaRa, 大连) 在荧光热循环仪 (Applied Biosystems, 美国) 上进行实时荧光定量PCR检测。将内参基因 *GAPDH* 做均一化分析。小鼠目的基因 *AGER*^[15]、*HMGB1*^[15]、*TLR-4*^[16]、白细胞介素 (interleukin, *IL*) -1 β ^[17]、*IL-6*^[17]、趋化因子配体2 (C-C motif chemokine ligand 2, *CCL2*)^[18]、肿瘤坏死因子 α (tumor necrosis factor- α , *TNF- α*)^[18]、干扰素 γ (interferon- γ , *IFN- γ*)^[19]和*IL-2*^[19]的引物序列见表1。

表1 引物序列

Table 1 Primer Sequence

目的基因 Target gene	上游引物(5'-3') Forward primer	下游引物(5'-3') Reverse primer
PVM	GCCTGCATCAACACAGTGTGT	GCCTGATGTGGCAGTGCTT
TLR-4	ATGGCATGGCTTACACCACC	GAGGCCAATTTGTCTCCACA
AGER	ACATGTGTGTCTGAGGGAAGC	AGCTCTGACCGCAGTGTAAG
HMGB1	CTTCGGCCTTCTTCTTGTCT	GGCAGCTTTCTTCTCATAGGG
IL-2	CCCAAGCAGGCCACAGAATTGAAA	AGTCAAATCCAGAACATGCCGCAG
IFN-γ	AGAGGATGGTTTGCATCTGGGTCA	ACAACGCTATGCAGCTTGTCGTG
CCL2	GGCTCAGCCAGATGCAGTTAA	CCTACTCATTGGGATCATCTTGCT
TNF-α	GACAAGGCTGCCCGACTA	TTTCTCCTGGTATGAGATAGCAAATC
IL-1β	ATGAGGACATGAGCACCT TC	CATTGAGTTGGAGAGCTTTC
IL-6	GACTTCATCCAGTTGCCTTC TT	TCCACGATTTCAGAGAACA

注：PVM，小鼠肺炎病毒；TLR-4，Toll样受体4；AGER，晚期糖基化终产物特异性受体；HMGB1，高迁移率族蛋白B1；IL-2，白细胞介素-2；IFN-γ，干扰素-γ；CCL2，趋化因子配体2；TNF-α，肿瘤坏死因子α。

Note: PVM, pneumonia virus of mice; TLR-4, toll-like receptor 4; AGER, advanced glycosylation end-product-specific receptor; HMGB1, high mobility group box 1; IL-2, interleukin-2; IFN-γ, interferon-γ; CCL2, C-C motif chemokine ligand 2; TNF-α, tumor necrosis factor-α.

1.5 数据分析

采用GraphPad Prism 9.5.1软件进行统计分析。各组数据均符合正态分布，以均值±标准差显示；采用单因素方差分析，然后用LSD-*t*检验进行组内两两比较。*P*<0.05为差异有统计学意义。

2 结果

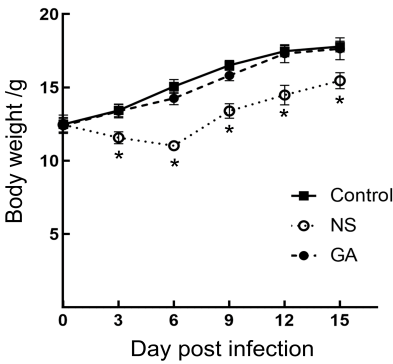
2.1 GA可显著减轻PVM感染引起的小鼠急性症状

通过外形观察评价小鼠的临床症状，结果显示，Control组小鼠无任何发病症状。与Control组相比，NS组小鼠在感染第3天(3 dpi)时体重下降，6 dpi时体重显著下降(*P*<0.05)，在9、12和15 dpi时体重仍呈下降趋势(*P*<0.05)；GA组无明显的临床症状，虽然出现一些体重变化，但差异无统计学意义(*P*>0.05)(图2)，提示GA能有效改善由PVM感染所致的小鼠体重下降症状。

2.2 GA能减轻PVM感染所致的小鼠肺组织病理损伤并抑制HMGB1的表达

苏木精-伊红染色结果显示：与Control组小鼠(图3A)相比，NS组小鼠肺组织出现肺泡间隔增厚、肺泡腔出血、血管充血现象(图3B)，GA治疗组小鼠肺组织中由PVM感染引起的病理症状明显得到改善(图3C)。

免疫组织化学检测结果显示：Control组小鼠肺组织中未检测到PVM阳性信号(图3D)，NS组小鼠肺组织中检测到较强的PVM阳性信号(图3E)；与NS组相



注：Control，未感染对照组；NS，小鼠肺炎病毒(PVM)感染后生理盐水(NS)处理组；GA，感染后(GA)甘草酸处理组。3组小鼠体重以平均值±SD表示(*n*=6)。与对照组相比，NS治疗组小鼠在感染后第3、6天时体重下降，9天后体重增加，与对照组相比仍有显著差异(*P*<0.05)；GA组小鼠与对照组相比无显著差异(*P*>0.05)。

Note: The body weights of mice in the three groups were expressed as mean value±SD (*n*=6). Compared with the Control group, the NS group experienced weight loss at 3 and 6 dpi. Although weight gain in the NS group could be detected after 9 dpi, there were significant differences compared to the Control group (*P*<0.05). There were no significant weight differences in GA group compared to the Control group (*P*>0.05). Control: uninfected control group; NS: group treated with normal saline (NS) after infection; GA: group treated with glycyrrhizic acid (GA) after infection.

图2 PVM感染后小鼠生长曲线

Figure 2 Body weight curves of mice after PVM infection

比，GA治疗组小鼠肺组织中PVM阳性信号明显减弱(图3F)，说明GA组小鼠肺组织中PVM病毒量低于NS组。

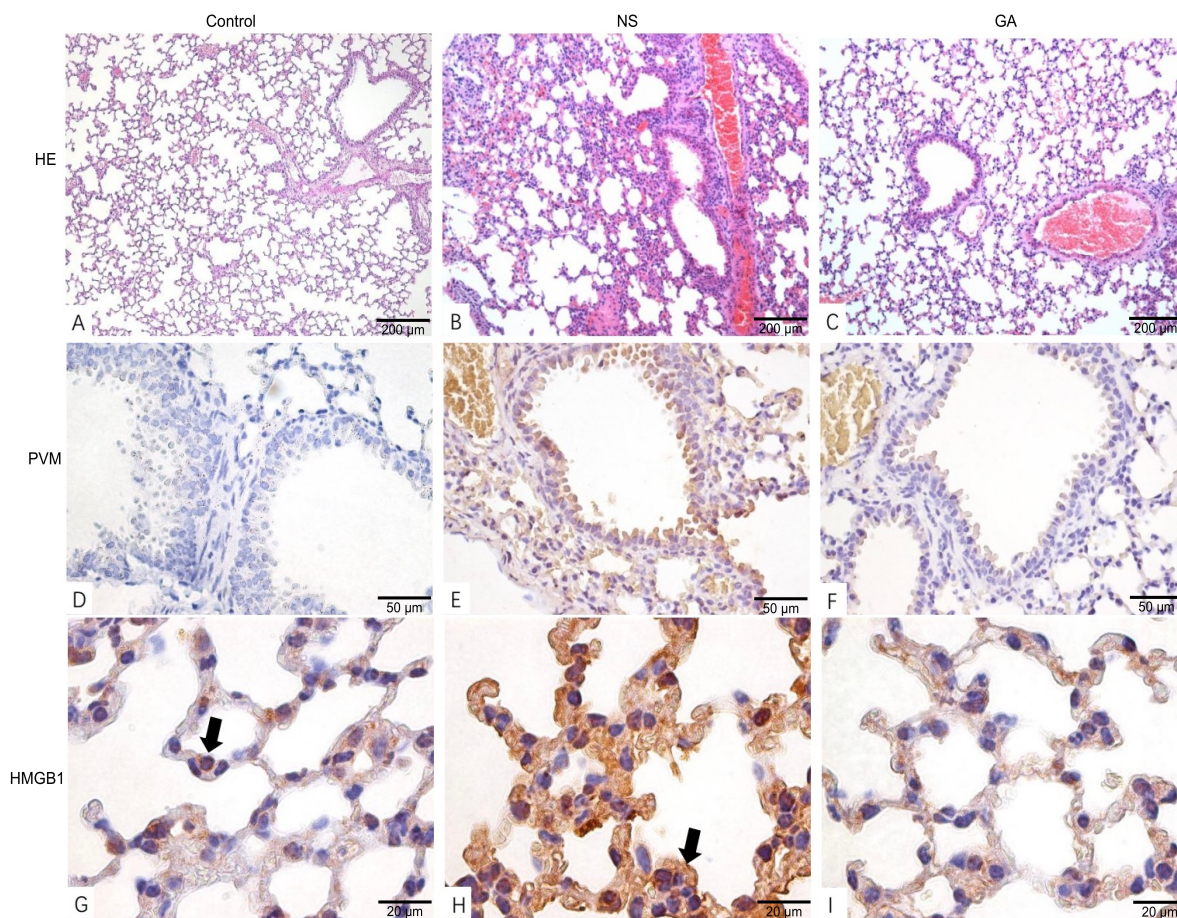
HMGB1可能是病毒感染引起的促炎因子，作为一种核结合因子，在没有病理损伤时多位于细胞核内。免疫组织化学检测结果显示：Control组小鼠肺组

织中 HMGB1 蛋白阳性信号主要出现在细胞核 (图 3G); NS 组小鼠肺组织中 HMGB1 蛋白阳性信号明显增强, 而且同时分布在细胞核和细胞质中 (图 3H); 而 GA 干预后, 小鼠肺组织中 HMGB1 蛋白阳性信号强度明显减弱 (图 3I), 提示 GA 能抑制小鼠肺组织中 HMGB1 的表达。

2.3 GA 能有效逆转 PVM 感染所致小鼠肺组织中 HMGB1 等表达水平的异常变化

实时荧光定量 PCR 结果显示: 与 Control 组相比, NS 组小鼠肺组织中 HMGB1 (图 4A)、IL-1 β (图 4G)

和 IL-2 (图 4I) 的转录水平均显著上调 ($P < 0.05$), 而 AGER (图 4B)、TLR-4 (图 4C)、TNF- α (图 4D)、CCL2 (图 4E)、IL-6 (图 4F) 和 IFN- γ (图 4H) 的表达水平没有显著变化 ($P > 0.05$); 与 NS 组相比, GA 组小鼠肺组织中, HMGB1 (图 4A)、IL-1 β (图 4G)、IFN- γ (图 4H) 和 IL-2 (图 4I) 的表达水平显著降低 ($P < 0.05$), 但 AGER 的表达水平显著增高 ($P < 0.05$) (图 4B), 提示 GA 能抑制 PVM 感染诱导的小鼠肺组织中 HMGB1、IL-1 β 、IFN- γ 和 IL-2 的表达, 并能促进肺组织中 AGER 的表达。

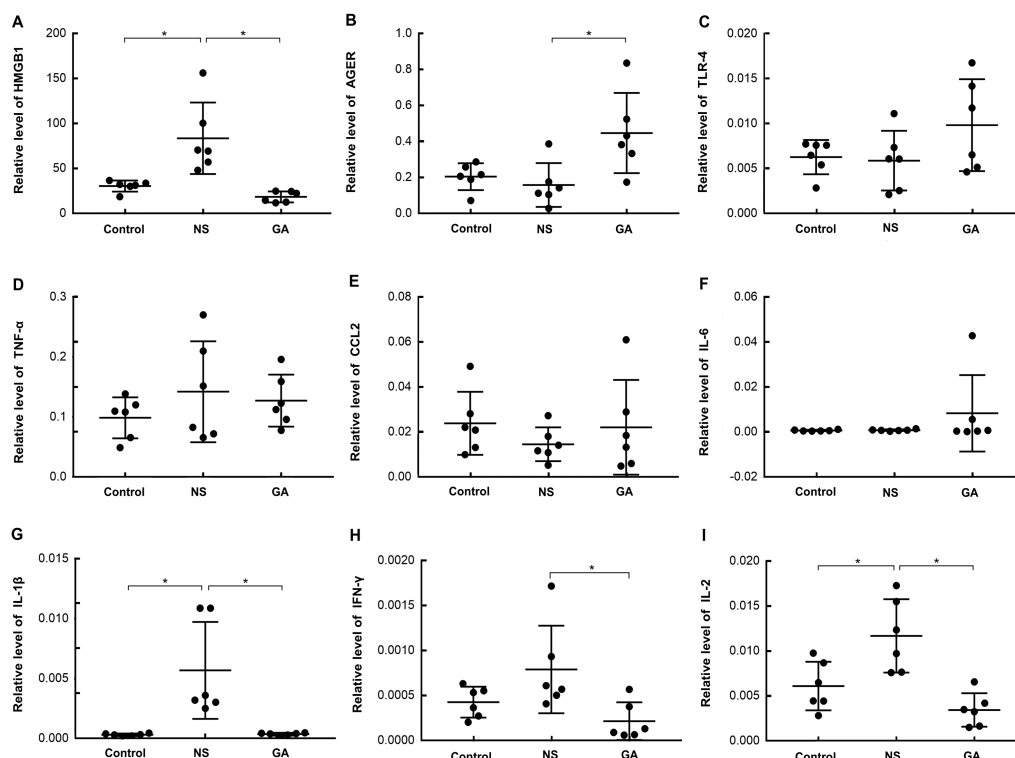


注: A ~ C, 组织病理学分析显示 PVM 感染后的肺组织病理变化 (苏木精-伊红染色, $\times 10$)。与 Control 组相比, NS 组小鼠肺泡间隔增厚, 肺泡腔出血, 血管充血; GA 组无明显的病理症状。D ~ F, 免疫组织化学检测结果显示 GA 组肺组织中 PVM 病毒载量低于 NS 组 (TMB 染色, $\times 40$)。G ~ I, 免疫组织化学分析显示 HMGB1 在肺组织中的表达 (TMB 染色, $\times 100$)。Control 组中箭头指示 HMGB1 在细胞核位置; NS 组可见 HMGB1 过表达, 且分布于细胞质。Control: 未感染对照组; NS: PVM 感染后生理盐水处理组; GA: PVM 感染后甘草酸处理组。

Note: A-C, Histopathological analysis showed the pulmonary tissue pathological changes after PVM infection (HE staining, $\times 10$). Compared with the Control group, thickened alveolar septa, hemorrhage in alveolar cavities, and vascular hyperemia were detected in the NS group; No significant pathological symptom was observed in the GA group. D-F, Immunohistochemical analysis demonstrated lower PVM viral load in pulmonary tissues in the GA group compared to the NS group (TMB staining, $\times 40$). G-I, Immunohistochemical analysis showed the HMGB1 expression in pulmonary tissues (TMB staining, $\times 100$). In the Control group, the cell nucleus locations of HMGB1 were indicated by arrows; in the NS group, HMGB1 was overexpressed and distributed in the cytoplasm. Control: uninfected control group; NS: group treated with normal saline (0.9% NaCl) after PVM infection; GA: group treated with glycyrrhizic acid after PVM infection. PVM: pneumonia virus of mice.

图 3 PVM 感染后小鼠肺部病理变化

Figure 3 Pathological changes in the lungs of mice after PVM infection in three groups



注：实时荧光定量PCR检测3组小鼠肺组织HMGB1、AGER、TLR-4、IL-1 β 、TNF- α 、CCL2、IL-6、IFN- γ 和IL-2相对于内参基因GAPDH的表达。 $n=6$, * $P<0.05$ 。Control: 未感染对照组; NS: PVM感染后生理盐水处理组; GA: PVM感染后甘草酸处理组。

Note: Real-time fluorescence quantitative PCR was performed on the pulmonary tissues from mice in three groups. The expression of high mobility group box 1 (HMGB1), advanced glycosylation end-product-specific receptor (AGER), Toll-like receptor 4 (TLR4), interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), C-C motif chemokine ligand 2 (CCL2), IL-6, interferon- γ (IFN- γ), and IL-2 were shown as ratios to GAPDH. $n=6$, * $P<0.05$. Control: uninfected control group; NS: group treated with normal saline (0.9%NaCl) after PVM infection; GA: group treated with glycyrrhizic acid after PVM infection. PVM: pneumonia virus of mice.

图4 PVM感染后小鼠肺组织中炎症因子与警报素HMGB1及其受体AGER的表达

Figure 4 Expression of inflammatory factors and the alarmin HMGB1 and its receptor AGER after PVM infection in pulmonary tissue

3 讨论

抗病毒的免疫应答是一把双刃剑。病毒入侵会暴露病原体相关分子模式 (pathogen-associated molecular pattern, PAMP), 这些模式可以被Toll样受体 (Toll-like receptors, TLRs) 等先天免疫系统受体识别, 刺激炎症因子的释放, 炎症因子可以吸引更多的炎症细胞。细胞因子的级联扩增将激活先天免疫和随后的适应性免疫来控制病毒感染。然而, 炎症反应也会导致组织损伤^[1]。

HMGB1是一种非组蛋白核蛋白, 根据其亚细胞定位不同具有多种功能。在细胞核中, HMGB1是维持染色体结构和功能的DNA伴侣; 在细胞质中, HMGB1可以通过与BECN1蛋白 (Beclin-1) 结合来促进自噬。在主动分泌或被动释放后, 细胞外的HMGB1通常作为

一种DAMPs分子, 可通过不同的受体结合或直接摄取, 从而调节炎症和免疫反应^[22]。HMGB1作为一种警报素, 可在哮喘患者和肺功能障碍患者中检测到^[21]。HMGB1释放后, 炎症细胞因子如IL-1 β 、IL-6、TNF- α 被激发, 这与组织病理损伤有关。细胞外的HMGB1主要与TLR-4和AGER结合, 引发炎症反应^[20]。已有研究证明, 通过阻断TLR4-HMGB1或AGER-HMGB1通路可成功抑制HMGB1依赖的炎症反应过程^[23]。

GA治疗可通过降低病毒感染或脂多糖 (lipopolysaccharide, LPS) 给药后支气管肺泡灌洗液中IL-1 β 、TNF- α 等炎症细胞因子的表达水平, 同时减少M1型和M2型巨噬细胞的表型标志物表达, 抑制巨噬细胞活化, 从而减轻肺组织病理学损伤^[24]。GA具有抑制病毒吸附和渗透、凝血酶和细胞外HMGB1分泌的

作用,已被证实是 HMGB1 的有效拮抗剂^[7]。除了感染诱导的炎症外,乙型肝炎病毒(hepatitis B virus, HBV)转基因小鼠还显示, HMGB1 在坏死炎症肝灶中从细胞核易位到细胞质, GA 治疗可以显著减少炎症细胞的募集^[25]。此外研究显示, GA 可通过 ACE2 的下游调节降低 TLR-4 表达,从而诱导抗炎活性^[26]。GA 可降低肺和心脏中 TLR 的表达,从而减少细胞因子的释放^[27-28],并在 TLR 介导的 ARDS 中显示出保护作用^[29]。

本研究在小鼠感染 PVM 后出现的肺脏炎症中,验证了肺脏病理损伤严重程度与小鼠肺组织内 HMGB1 过表达及其从细胞核迁移至细胞质有关。给予 GA 治疗后,肺脏病理损伤明显减轻,炎症因子表达水平降低, HMGB1 的过表达被抑制,小鼠感染 PVM 后体重下降的临床症状也被抑制。这些结果提示, GA 能控制病毒感染后的炎症反应,特别是能抑制 HMGB1 过表达和细胞内转移,这对治疗病毒性肺炎具有重要意义。但与已有结果相悖的是,在我们的研究中发现,在感染后,给药的小鼠虽然 HMGB1 的表达降低,向胞质位移的情况被抑制,但 HMGB1 的受体,同时也是诱发炎症的受体 TLR-4 和 AGER 表达量上调。AGER 表达水平相较于感染组和对照组显著升高, TLR-4 的表达水平也有升高的趋势。推测可能的原因是 HMGB1 在被 GA 抑制后细胞的代偿性提高受体的表达。理论上炎症因子受体表达的上升将对肺炎的治疗产生不利的影响,而事实是我们在实验中观察到给予 GA 治疗的小鼠肺部病理损伤明显减轻。在本研究中, GA 对于 PVM 感染后小鼠肺损伤的改善效果是由哪一种药理作用产生的,什么分子在抗病毒反应中发挥主要作用,以及 GA 是通过怎样的机制来抑制 HMGB1,这一系列问题有待更进一步的探索。

[医学伦理声明 Medical Ethics Statement]

本研究涉及的所有动物实验均已通过中国医学科学院医学实验动物研究所实验动物使用与管理委员会批准(批件号: XZG18001)。所有实验过程均遵照中国实验动物相关法律法规条例要求进行。

All experimental animal protocols in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the Chinese Institute of Laboratory Animal Science (Approval Letter No. XZG18001). All experimental procedures were performed in accordance with the requirements of laws and regulations in China related to experimental animals, including *Animal Management Regulations* (01/03/2017) and *Laboratory*

Animal: Guideline for Ethical Review of Animal Welfare (GB/T 35892-2018).

[作者贡献 Author Contribution]

刘芸负责实验操作、论文撰写、统计分析;
冯婷婷、佟巍、郭智、李夏参与了动物实验工作;
孔琪参与了统计分析工作;
向志光负责课题整体构思和文章修改工作。

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所有作者均声明不存在利益冲突。

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