

DOI:10.3724/SP.J.1008.2012.00818

雌马酚对芥子气所致家兔皮肤损伤的治疗作用及其机制的初步研究

郑庆虎¹, 于蕊², 刘强¹, 赵静¹, 徐立³, 周欣¹, 孙本全¹, 高莉⁴, 梁晓博^{5*}, 吴建华^{1*}

1. 第二军医大学长海医院皮肤科, 上海 200433
2. 解放军 71320 部队门诊部皮肤科, 开封 475003
3. 第二军医大学长海医院急诊科, 上海 200433
4. 第二军医大学长海医院病理科, 上海 200433
5. 解放军 309 医院皮肤科, 北京 100091

[摘要] **目的** 探讨具有雌激素样活性的雌马酚 (equol, Eq) 对芥子气染毒皮肤创面愈合的影响及可能的机制。**方法** 以芥子气 (0.5 mg/cm²) 染毒家兔臀部皮肤为实验模型, 染毒创面连续 1 周外用不同浓度 (10、50、100 μmol/L) 的 Eq 后, 观察创面外观变化; 取创面活检标本观察组织病理学变化; 用流式细胞术分析细胞凋亡、DNA 周期变化; 用试剂盒检测染毒创面中 IL-6、TNF-α 的浓度。**结果** 染毒 120 h 后, 所有治疗组染毒创面红斑、水肿、糜烂、坏死等皮损评分低于染毒对照组 ($P < 0.05$); 染毒 168 h 后, 50 μmol/L 治疗组染毒创面组织病理学炎症、坏死等改变评分低于染毒对照组 ($P < 0.05$), 各治疗组细胞凋亡百分比低于染毒对照组 ($P < 0.05$), 各治疗组细胞 DNA (S+G₂-M) 周期百分比高于染毒对照组 ($P < 0.05$), 治疗组细胞中的 IL-6、TNF-α 含量低于染毒对照组 ($P < 0.05$)。**结论** Eq 可能通过抑制皮损局部炎症反应减少细胞凋亡, 增强细胞增殖能力, 从而促进芥子气所致皮肤损伤的愈合。

[关键词] 芥子气; 中毒; 接触性皮炎; 雌马酚**[中图分类号]** R 758.2**[文献标志码]** A**[文章编号]** 0258-879X(2012)08-0818-06

Therapeutic effect of equol on local skin wounds caused by mustard gas in rabbits and related mechanism

ZHENG Qing-hu¹, YU Rui², LIU Qiang¹, ZHAO Jing¹, XU Li³, ZHOU Xin¹, SUN Ben-quan¹, GAO Li⁴, LIANG Xiao-bo^{5*}, WU Jian-hua^{1*}

1. Department of Dermatology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
2. Department of Dermatology, No. 71320 Troop of PLA, Kaifeng 475003, Henan, China
3. Department of Emergency, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
4. Department of Pathology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
5. Department of Dermatology, No. 309 Hospital of PLA, Beijing 100091, China

[Abstract] **Objective** To study the therapeutic effect of estrogenic equol (Eq) on mustard gas-caused skin wounds and the possible mechanism. **Methods** A rabbit skin wound model was established by contaminating the hip skin with mustard gas (0.5 mg/cm²). The contaminated wound was treated externally with different concentrations (10, 50, and 100 μmol/L) of Eq for a week, and the changes of the wound were observed macroscopically. Wound specimens were collected for histopathological evaluation. Cell apoptosis and DNA cycle changes were analyzed by flow cytometry. Levels of IL-6 and TNF-α in the contaminated wound specimens were detected. **Results** The scores of erythema, edema, erosion and necrosis of the contaminated wounds in the treatment groups were significantly lower than those in the control group 120 h after mustard gas exposure ($P < 0.05$). The scores for changes of histopathological inflammation and necrosis were significantly lower in the 50 μmol/L Eq treatment group than those in the control group 168 h after mustard gas exposure ($P < 0.05$). The results of flow cytometry showed that the proportion of apoptotic cells in the control group was significantly higher than those in the treatment groups 168 h after mustard gas exposure ($P < 0.05$), and the proportion of cells in DNA (S+G₂-M) phase in the treatment groups was significantly higher than that in the control group ($P < 0.05$). The levels of IL-6 and TNF-α in the treatment groups

[收稿日期] 2012-04-15**[接受日期]** 2012-06-28**[基金项目]** 国家科技部重大专项 (2009ZXJ09004-075, 2011ZXJ09102-02B), 第二军医大学长海医院“1255 计划”项目。Supported by the Major National Science and Technology Project of China (2009ZXJ09004-075, 2011ZXJ09102-02B), and “1255 Project” of Changhai Hospital.**[作者简介]** 郑庆虎, 硕士, 主治医师。E-mail: zhengqinghu205@163.com

* 通信作者 (Corresponding authors). Tel: 010-66775473, E-mail: liangxiaobo309@yahoo.cn; Tel: 021-31161562, E-mail: wujh_ch@163.com

were significantly lower than those in the control group 168 h after mustard gas exposure ($P < 0.05$). **Conclusion** Eq can promote healing of mustard gas-caused local skin damaged by inhibiting inflammatory response and cell apoptosis and enhancing cell proliferation.

[Key words] mustard gas; poisoning; contact dermatitis; equol

[Acad J Sec Mil Med Univ, 2012, 33(8): 818-823]

芥子气是一种糜烂性毒剂,具有性质稳定、作用持久、致伤广泛、防护与消毒困难以及无特效抗毒剂的特点。芥子气的这些特性使其在军事医学中占有重要地位。皮肤既是芥子气染毒的主要途径,又是主要靶器官,所以芥子气皮肤染毒的防治一直是军事医学研究的重点。雌马酚(equol, Eq)是大豆异黄酮的代谢产物之一,具有抗氧化应激、阻止血管内皮损伤、对抗炎症介质等功能^[1-2]。研究显示,10~15 $\mu\text{mol/L}$ 是Eq的体内有效浓度;在培养条件下,10 $\mu\text{mol/L}$ Eq没有细胞毒性^[2]。本研究观察了不同浓度Eq对芥子气所致家兔皮肤损伤创面愈合的情况,以探讨Eq对芥子气染毒皮肤创面愈合的影响及可能的机制。

1 材料和方法

1.1 实验动物及分组、备皮 选用20只雄性、体重为2 000~2 300 g的新西兰大白兔(购自中国科学院上海实验动物中心)于第二军医大学实验动物中心适应性喂养7 d,观察饮水和进食情况,确定无异常情况后将其随机分成4组(每组5只),即:染毒对照组、治疗1组、治疗2组和治疗3组;用剃须刀在20只新西兰大白兔右侧臀部剃毛,用记号笔划出边长为2 cm的正方形实验区,待2 d后染毒备用。

1.2 试剂及配制 纯度 $\geq 99.0\%$ 的Eq购于Sigma公司(批号:44405),于第二军医大学长海医院药理学部用生理盐水配制成3份,浓度分别为10、50和100 $\mu\text{mol/L}$,置于4℃的冰箱中冷藏待用。

1.3 染毒模型建立及给药方法 将染毒对照组及治疗组共20只大白兔运送至第二军医大学海军医学系防化医学教研室,用取样枪在大白兔标记区外涂质量浓度为0.1 mg/ μl 的芥子气母液20 μl (0.5 mg/ cm^2),然后迅速送回至第二军医大学实验动物中心饲养待用。染毒1 h后,分别给治疗1组、治疗2组、治疗3组的大白兔染毒区外涂浓度为10、50和100 $\mu\text{mol/L}$ 的Eq 200 μl ,每隔24 h按同样方法给药一次,共7次。染毒对照组不作任何处理。

1.4 观察指标及评分 每天外涂药物后,分别给染毒标记区拍照,记录染毒对照组和各治疗组染毒创面的红斑、水肿、糜烂、坏死等4种皮损变化情况。参照文献[3]根据每种皮损所占染毒创面的面积百

分比,分别在染毒24、72、120、168 h后给每个创面皮损进行评分(皮损面积 $\leq 25\%$ 为1分,皮损面积占26%~74%为2分,皮损面积 $\geq 75\%$ 为3分;单项最高评分是3分,总分最高评分是12分)。

染毒168 h后,取创面组织活检标本做病理切片,用H-E染色法和弹力、胶原纤维双重组合染色法观察创面病理变化。通过细胞凋亡百分比、DNA周期变化及炎症细胞因子IL-6、TNF- α 检测,观察创面炎症变化和愈合增殖情况。根据文献[4]关于芥子气对皮肤损伤创面病理变化特点,结合染毒创面病理切片其表皮结构完整性及表皮真皮内有无炎症渗出、毛细血管充血扩张、局灶性坏死、炎症细胞浸润等情况,制定染毒创面病理损害评分标准(表1)。按评分标准,分别对各组创面病理切片进行评分。

1.5 流式细胞法检测细胞凋亡 染毒168 h后,用20%的尿素酯按5 mg/kg剂量沿大白兔耳缘静脉注射,在麻醉状态下分离1/4标记区皮肤,用机械法将皮肤组织剪碎,用200目尼龙网过滤成单细胞悬液;置于柠檬酸缓冲液1 h以上;调整细胞密度至 $1 \times 10^6/\text{ml}$ 左右;离心后去除上清液;加入1 800/ μl A溶液(胰酶消化液)充分作用;加入1 500/ μl B溶液(胰酶抑制酶液、RNase)2 h;加入1 500/ μl C溶液(PI-碘化丙啶)作用15 min以上;用200目尼龙网再次过滤;离心去除上清液,加75%的乙醇1 ml,振混放入4℃的冰箱中过夜,次日离心倒掉乙醇,然后加入1 ml PBS,振匀离心后去除上清液;上机进行DNA细胞周期和凋亡亚倍体采样分析。

1.6 细胞炎症因子IL-6、TNF- α 检测 按上述方法分离1/4标记区皮肤,用机械法将皮肤组织剪碎,用200目尼龙网过滤成单细胞悬液,分装后放入-20℃的冰箱中冷冻保存;实验前20 min从冰箱中取出试剂盒,平衡至室温(20~25℃);取出所需数量的板条,设标准孔8孔,在待测孔中每孔各加入待测样品100 μl ;将反应板置37℃ 120 min;用洗涤液将反应板充分洗涤4~6次,并在滤纸上印干;每孔中加入第一抗体工作液50 μl ;将反应板充分混匀后置37℃ 60 min;用洗涤液将反应板充分洗涤4~6次后在滤纸上印干;每孔加酶标抗体工作液100 μl ;将反应板置37℃ 60 min;在每孔中加入底物液100 μl ,置37℃暗处反应5~10 min;每孔加入50 μl 终止液混

匀;在 450 nm 处测光密度,检测细胞炎症因子 IL-6、TNF- α 的浓度。IL-6、TNF- α 试剂盒由上海森雄科技公司从美国 R&G 公司进口分装。

1.7 统计学处理 采用 SPSS 18.0 统计软件对数据进行统计分析,实验参数值均用 $\bar{x} \pm s$ 表示,组间比较采用单因素方差分析,检验水平(α)为 0.05。

表 1 染毒创面病理改变的评分标准

Tab 1 Assessment standards for the pathological changes of infected wounds

Score	Epidermal structure	Epidermis inflammation degree	Dermis inflammation degree
0	Normal epidermis, clear construction	No exudates or inflammatory cells in epidermis	No inflammatory cells in the dermis
1	Almost normal epidermis, not piled with exudates or necrosis	Small amount of exudates in local epidermis, no obvious inflammation	Local infiltration of inflammatory cells around hair follicles, or capillary congestion and dilation in the dermis
2	Impaired epidermis, intermittent full-thickness necrosis, partial necrosis, covered with exudates	More infiltration of inflammatory cells within the epidermis, or significant exudates, or have one or more blisters	Scattered infiltration of inflammatory cells in the dermis, or no obvious infiltration of inflammatory cells, but more in certain areas
3	Full-thickness necrosis of the entire epidermis, the hierarchy disappears, covered with necrosis and exudates	Dense infiltration of inflammatory cells in necrotic epidermis	Diffuse infiltration of inflammatory cells in the dermis, or dense infiltration in certain areas
4	Entire epidermis necrosis before taking specimen	Entire epidermis necrosis before taking specimen	Dense infiltration of inflammatory cells in the dermis, or partial necrosis in deep dermis

2 结果

2.1 染毒创面皮肤评分结果 按创面皮肤评分标准,各组染毒创面在染毒不同时间后的评分结果见表 2。治疗 2 组的创面损害评分在染毒 72 h 后即低

于染毒对照组,且持续至染毒 168 h,差异均有统计学意义($P < 0.05$);治疗 1 组和治疗 3 组的创面损害评分均于染毒 120 h 时低于染毒对照组,差异有统计学意义($P < 0.05$)。提示 Eq 能减轻芥子气所致染毒创面的损害、促进创面的愈合。

表 2 不同时间染毒创面损害的评分比较

Tab 2 Scores of mustard gas-contaminated wounds at different time points

$n=5, \bar{x} \pm s$

Group	Time of contamination t/h			
	24	72	120	168
Contaminated	2.00 \pm 1.00	2.60 \pm 1.14	4.20 \pm 1.79	4.00 \pm 2.35
Treatment group 1	1.80 \pm 0.48	2.20 \pm 0.45	1.60 \pm 0.89*	1.40 \pm 0.89*
Treatment group 2	1.80 \pm 0.84	1.40 \pm 0.58*	1.20 \pm 0.45*	0.80 \pm 0.45*
Treatment group 3	1.80 \pm 1.30	1.60 \pm 0.89	1.40 \pm 1.14*	1.20 \pm 0.84*

Treatment group 1: 10 μ mol/L Eq; Treatment group 2: 50 μ mol/L Eq; Treatment group 3: 100 μ mol/L Eq. * $P < 0.05$ vs contaminated group

2.2 染毒创面组织病理学结果 各组染毒 168 h 后创面组织的 H-E 染色结果见图 1。染毒创面病理损害评分:染毒对照组为(9.00 \pm 0.71)分,治疗 1 组为(6.60 \pm 2.88)分,治疗 2 组为(5.60 \pm 2.61)分,治疗 3 组为(5.80 \pm 3.11)分。治疗 2 组病理损害评分最低,与染毒对照组之间差异有统计学意义($P < 0.05$)。提示 Eq 能减轻芥子气所致染毒创面的组织病理学改变、促进创面的愈合。

2.3 染毒创面组织弹力、胶原纤维双重染色结果 染毒 168 h 各组创面组织经弹力、胶原纤维双

重染色后显示,染毒对照组胶原纤维明显肿胀变性,弹力纤维断裂明显,而治疗 2 组胶原纤维肿胀明显较染毒对照组减轻,弹力纤维断裂较染毒对照组减少(图 2),提示治疗组皮损组织的胶原、弹力纤维损害较轻。

2.4 细胞凋亡百分比变化 由图 3 可见,治疗 2 组细胞凋亡百分比最低,但各治疗组间比较差异无统计学意义。与染毒对照组比较,各治疗组细胞凋亡百分比均降低($P < 0.05$),提示治疗组皮损组织中细胞凋亡减少、增殖能力增强。

2.5 染毒创面细胞 DNA 周期百分比变化 由表 3 可见, 各治疗组细胞 DNA 周期中 S+G₂-M 百分比均高于染毒对照组 ($P < 0.05$), 其中治疗 2 组最高; 各治疗组间细胞 DNA 周期中 S+G₂-M 百分比差异无统计学意义, 提示治疗组皮损组织中细胞增殖能力增强。

2.6 标记区细胞炎症因子 IL-6、TNF- α 的变化 由表 4 可见, 与染毒对照组比较, 3 个治疗组的细胞因子 IL-6、TNF- α 含量均降低, 差异有统计学意义 ($P < 0.05$), 其中治疗 3 组细胞因子 IL-6、TNF- α 含量最低, 各治疗组间比较差异无统计学意义。

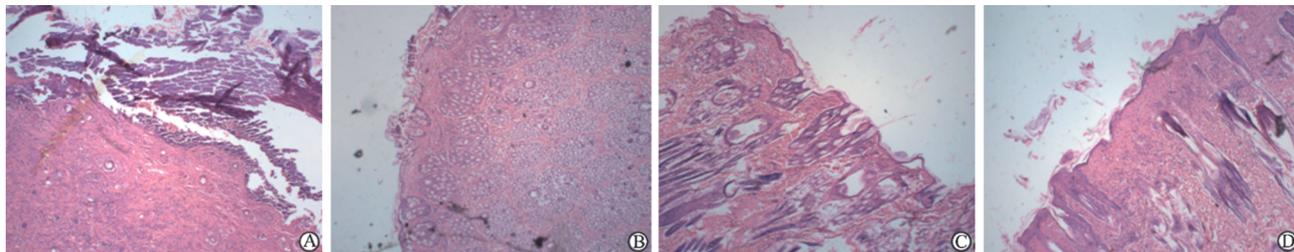


图 1 染毒 168 h 后各组创面组织 H-E 染色结果

Fig 1 Histopathological changes of wounds 168 h after contamination in each group (H-E staining)

A: Contaminated group, necrosis of the whole epidermis, piled with large amounts of inflammatory exudates, with local hemorrhage; lots of inflammatory cells were seen in the dermis; pathological score 9. B: Treatment group 1 (10 $\mu\text{mol/L}$ Eq), partial necrosis of epidermis, piled with large amounts of inflammatory exudates, with moderate inflammatory cells in the dermis; pathological score 6. C: Treatment group 2 (50 $\mu\text{mol/L}$ Eq), normal epidermis, no exudates, inflammatory cells, necrosis, or congestion and dilation of capillaries; with obvious congestion of capillaries in the dermis, clear local infiltration of inflammatory cells; pathological score 2. D: Treatment group 3 (100 $\mu\text{mol/L}$ Eq), impaired integrity of epidermis, partial necrosis, with no exudates, inflammatory cells in the epidermis; with obvious congestion of capillaries in the dermis, diffuse infiltration of inflammatory cells; pathological score 4. Original magnification: $\times 40$

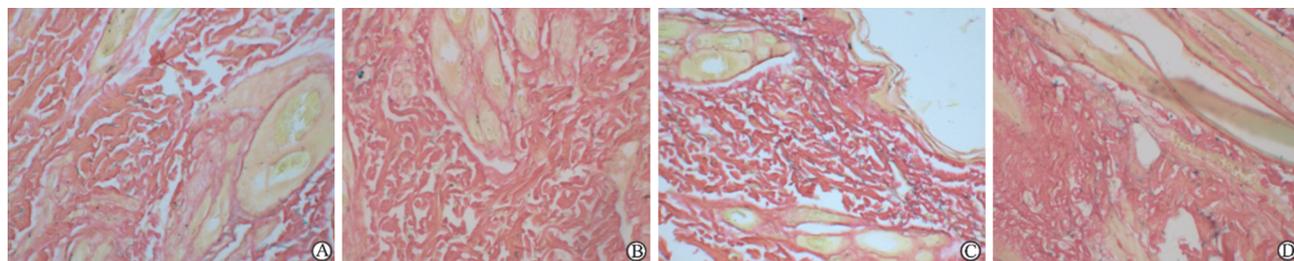


图 2 染毒 168 h 后各组创面组织弹力、胶原纤维双重染色结果

Fig 2 Histopathological changes of wounds 168 h after contamination in each group (double staining)

A: Contaminated group, with distinct collagen edema and degeneration, breakage of elastic fibers; B: Treatment group 1 (10 $\mu\text{mol/L}$ Eq), less collagen edema compared with contaminated group, but still with obvious breakage of elastic fibers; C: Treatment group 2 (50 $\mu\text{mol/L}$ Eq), obviously decreased collagen edema and breakage of elastic fibers; D: Treatment group 3 (100 $\mu\text{mol/L}$ Eq), partial collagen edema was relieved compared with the contaminated group, but still with obvious breakage of elastic fibers. Original magnification: $\times 40$

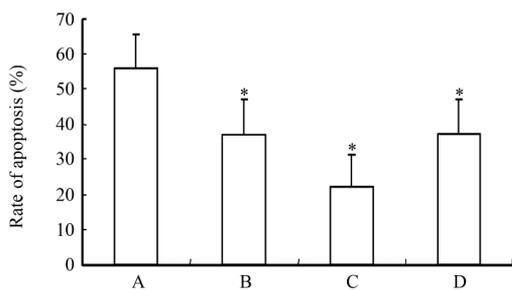


图 3 各组细胞凋亡百分比比较

Fig 3 Comparison of cell apoptosis percentage in each group

A: Contaminated group; B: Treatment group 1 (10 $\mu\text{mol/L}$ Eq); C: Treatment group 2 (50 $\mu\text{mol/L}$ Eq); D: Treatment group 3 (100 $\mu\text{mol/L}$ Eq). * $P < 0.05$ vs contaminated group; $n = 5$, $\bar{x} \pm s$

表 3 染毒创面细胞 DNA 周期百分比变化比较

Tab 3 Comparison of DNA cycle change of cells from contaminated wounds

Group	DNA cycle		
	S	G ₂ -M	S+G ₂ -M
Contaminated	7.33 \pm 2.29	3.01 \pm 0.99	10.34 \pm 2.99
Treatment group 1	15.37 \pm 4.59	0.78 \pm 1.19	16.16 \pm 5.07*
Treatment group 2	16.02 \pm 2.62	1.93 \pm 1.25	17.95 \pm 3.02*
Treatment group 3	13.59 \pm 3.55	2.27 \pm 0.92	14.87 \pm 3.06*

Treatment group 1, 2, 3: 10 $\mu\text{mol/L}$ Eq, 50 $\mu\text{mol/L}$ Eq, and 100 $\mu\text{mol/L}$ Eq, respectively. * $P < 0.05$ vs contaminated group

表 4 染毒创面细胞炎症因子 IL-6 和 TNF- α 的含量变化

Tab 4 Changes in contents of IL-6 and TNF- α in contaminated wounds

$n=5, \bar{x} \pm s, \rho_B / (\text{pg} \cdot \text{ml}^{-1})$

Group	IL-6	TNF- α
Contaminated	689.27 \pm 766.11	588.62 \pm 599.35
Treatment group 1	187.16 \pm 169.18*	253.24 \pm 143.11*
Treatment group 2	71.98 \pm 27.53*	120.93 \pm 55.41*
Treatment group 3	44.83 \pm 15.29*	83.80 \pm 17.36*

Treatment group 1, 2, 3: 10 $\mu\text{mol/L}$ Eq, 50 $\mu\text{mol/L}$ Eq, and 100 $\mu\text{mol/L}$ Eq, respectively. * $P < 0.05$ vs contaminated group

3 讨论

芥子气(sulfur mustard)又称 β, β' -二氯二乙基硫醚,难溶于水,易溶于有机溶剂,是一种典型的双功能烷化剂。细胞内许多重要成分含有 S、N、O 等亲核中心,是芥子气的靶部位,生理条件下,芥子气与体内许多亲核性基因如氨基、巯基、羟基、磷酸基及咪唑基等反应,因此芥子气具有广泛且复杂的生物学作用。

皮肤是芥子气中毒的主要途径,是易受损伤的重要部位,芥子气对皮肤的损伤作用主要是由于它对 DNA 烷化作用的结果^[5]。因 DNA 对芥子气极为敏感^[6],可使 DNA 的半保留复制被抑制,影响细胞分裂,使上皮细胞基底层被破坏,造成皮肤起疱、坏死、糜烂等^[7]。皮肤芥子气中毒机制虽然早有研究,但迄今尚未完全阐明,其生化假说一直是研究的热点,即 DNA 烷化损伤后,诱导修复和细胞凋亡,从而激活多聚(ADP-核糖)聚合酶(染色体酶),该酶利用烟酰胺腺嘌呤二核苷酸(NAD)为基质合成多聚 ADP-核糖,因而大量消耗细胞 NAD^+ ;细胞 NAD^+ 含量降低抑制了糖酵解,并使磷酸己糖旁路活化,导致蛋白酶释放,最终发生皮肤炎症、坏死等病理改变^[8]。也有研究认为芥子气的毒性与亲电子应激(ES)或氧化应激(OS)以及巯基水平包括谷胱甘肽(GT)的耗竭有关^[7]。

目前临床上对于芥子气所致皮肤损伤尚无公认的治疗措施,而一些具有甾体激素样活性的天然化学物质(如具有糖皮质激素样活性的人参皂苷^[9]和具有雌激素样活性的 Eq、染料木素、大豆苷元和葛根素^[10]等)正是通过减少皮肤氧化损伤、抑制皮肤局部炎症反应以及增强皮肤增殖能力等生物学效应而发挥皮肤防护作用^[11]。尤其是 Eq 作为多酚,是氢供体(电子供体),可以清除自由基^[1],具有明确的抗氧化应激^[1-2,12]、阻止血管内皮损伤、对抗炎症介质、一定浓度下上调内皮型一氧化氮合酶

(eNOS)^[2,13-14]、减少炎症状态下局部超氧负离子产物,从而改善局部血液循环、增强抗修复能力等作用^[2]。为了研究 Eq 对芥子气染毒皮肤创面愈合的影响及可能的机制,本研究根据文献^[15]选用家兔作为实验模型,通过对大白兔染毒对照组与治疗组的皮损变化情况和组织病理的形态改变情况比较,证实了 Eq 治疗组较染毒对照组愈合时间早、组织修复快。

芥子气中毒的病理机制也有可能和蛋白酶活化有关,如弹性蛋白酶、胰蛋白酶和类胰蛋白酶等^[16]。芥子气皮损中许多细胞可产生蛋白酶,有证据表明这些蛋白酶能水解真皮结缔组织,导致组织崩解和愈合延迟^[17]。胶原纤维和弹力纤维是真皮结缔组织的重要成分,因此通过观察这两种纤维的改变可在一定程度上反映创面的早期病变和后期的愈合状况。本实验采用这两种纤维的双重组合特殊染色法,观察到 Eq 治疗组的胶原纤维和弹力纤维病变较轻,恢复较快,提示 Eq 经创面吸收后可在一定程度上减少芥子气对真皮结缔组织的损伤并促进组织重建。

细胞在分裂增殖前,首先必须进行 DNA 的合成,即进入细胞周期的 S 期,合成后再进入细胞周期的 G₂ 期和 M 期。因此 S+G₂-M 期的比例增加,提示组织中细胞的增殖能力提高。本实验通过流式细胞分析,观察到 Eq 治疗组染毒创面中细胞凋亡百分比低于染毒对照组($P < 0.05$)、S+G₂-M 期细胞比例高于染毒对照组($P < 0.05$),提示 Eq 对于芥子气染毒皮损的细胞增殖和创面修复具有明显的促进作用,能显著抑制芥子气染毒局部皮肤细胞的凋亡。

IL-6 是一种多功能细胞因子,既可由淋巴细胞产生,也能由非淋巴细胞合成,在体内免疫反应调节、防御机制和急性期反应中起重要作用。IL-6 含量升高,多见于急性期蛋白的出现,与多种临床疾病有关,如急性炎症、烧伤等,可作为炎症反应的早期指标^[18-19]。TNF- α 前体以跨膜蛋白的形式存在于细胞内,在细胞外被剪切成相对分子质量为 17 000 的成熟形态(157 个氨基酸)。有活性的 TNF- α 以三聚体的形式存在。很多细胞都能合成 TNF- α ,包括各种免疫细胞(单核细胞、巨噬细胞、T 细胞、B 细胞、粒细胞等)和非免疫细胞。TNF- α 不仅能调节肿瘤细胞的生长,对很多细胞的生长、分化和代谢都有影响,血清 TNF- α 增高可见于肿瘤、炎症和免疫性疾病等^[20-21]。本实验发现 Eq 治疗组皮损区细胞中 IL-6、TNF- α 浓度低于染毒对照组($P < 0.05$),说明 Eq 治疗组的皮损炎症反应明显低于染毒对照组,具有明显的抗炎作用。

本实验结果显示 E_q 对于芥子气所致皮肤损伤的愈合具有明显的促进作用, 可使得染毒局部皮肤组织炎症细胞因子含量减少, 剂量依赖性地提高芥子气染毒局部皮肤组织细胞的增殖能力, 并抑制芥子气染毒局部皮肤细胞凋亡。因此, E_q 对于芥子气的皮肤中毒防治具有良好的应用前景。

4 利益冲突

所有作者声明本文不涉及任何利益冲突。

[参考文献]

- [1] Ma Y, Sullivan J C, Schreihofer D A. Dietary genistein and equol (4',7 isoflavandiol) reduce oxidative stress and protect rats against focal cerebral ischemia[J]. *Am J Physiol Regul Integr Comp Physiol*, 2010, 299: R871-R877.
- [2] Cheng C, Wang X, Weakley S M, Kougiaris P, Lin P H, Yao Q, et al. The soybean isoflavonoid equol blocks ritonavir-induced endothelial dysfunction in porcine pulmonary arteries and human pulmonary artery endothelial cells[J]. *J Nutr*, 2010, 140: 12-17.
- [3] 吴建华, 吕挺, 顾军, 朱明学. 不同复方软膏制剂对家兔芥子气皮肤染毒的预防作用[J]. *第二军医大学学报*, 2006, 27: 346-348.
- Wu J H, Lü T, Gu J, Zhu M X. Preventive effect of ointment preparations on sulfur mustard-induced skin damage in rabbits [J]. *Acad J Sec Mil Med Univ*, 2006, 27: 346-348.
- [4] 吴建华, 吕挺, 朱明学, 廖万清. 不同药物对猪皮肤芥子气损伤创面愈合的影响[J]. *海军医学杂志*, 2001, 22: 193-195.
- [5] Rikimaru T, Nakamura M, Yano T, Beck G, Habicht G S, Rennie L L, et al. Mediators, initiating the inflammatory response, released in organ culture by full-thickness human skin explants exposed to the irritant, sulfur mustard[J]. *J Invest Dermatol*, 1991, 96: 888-897.
- [6] Smith K J, Hurst C G, Moeller R B, Skelton H G, Sidell F R. Sulfur mustard: its continuing threat as a chemical warfare agent, the cutaneous lesions induced, progress in understanding its mechanism of action, its long-term health effects, and new developments for protection and therapy[J]. *J Am Acad Dermatol*, 1995, 32: 765-776.
- [7] Smith K J. The prevention and treatment of cutaneous injury secondary to chemical warfare agents. Application of these findings to other dermatologic conditions and wound healing[J]. *Dermatol Clin*, 1999, 17: 41-60.
- [8] Balali-Mood M, Hefazi M. The pharmacology, toxicology, and medical treatment of sulphur mustard poisoning[J]. *Fundam Clin Pharmacol*, 2005, 19: 297-315.
- [9] Ling C, Li Y, Zhu X, Zhang C, Li M. Ginsenosides may reverse the dexamethasone-induced down-regulation of glucocorticoid receptor[J]. *Gen Comp Endocrinol*, 2005, 140: 203-209.
- [10] Tang X, Zhu X, Liu S, Nicholson R C, Ni X. Phytoestrogens induce differential estrogen receptor beta-mediated responses in transfected MG-63 cells[J]. *Endocrine*, 2008, 34: 29-35.
- [11] Widyarini S. Protective effect of the isoflavone equol against DNA damage induced by ultraviolet radiation to hairless mouse skin[J]. *J Vet Sci*, 2006, 7: 217-223.
- [12] Rüfer C E, Kulling S E. Antioxidant activity of isoflavones and their major metabolites using different *in vitro* assays[J]. *J Agric Food Chem*, 2006, 54: 2926-2931.
- [13] Siow R C, Li F Y, Rowlands D J, de Winter P, Mann G E. Cardiovascular targets for estrogens and phytoestrogens: transcriptional regulation of nitric oxide synthase and antioxidant defense genes[J]. *Free Radic Biol Med*, 2007, 42: 909-925.
- [14] Mann G E, Bonacasa B, Ishii T, Siow R C. Targeting the redox sensitive Nrf2-Keap1 defense pathway in cardiovascular disease: protection afforded by dietary isoflavones[J]. *Curr Opin Pharmacol*, 2009, 9: 139-145.
- [15] 吴建华, 吕挺, 朱明学. 芥子气皮肤损伤不同动物模型的比较[J]. *第二军医大学学报*, 2006, 27: 223-224.
- Wu J H, Lü T, Zhu M X. Sulfur mustard induced skin damage: a comparison of different animal models[J]. *Acad J Sec Mil Med Univ*, 2006, 27: 223-224.
- [16] Papirmeister B, Gross C L, Meier H L, Petrali J P, Johnson J B. Molecular basis for mustard-induced vesication[J]. *Fundam Appl Toxicol*, 1985, 5: S134-S149.
- [17] Smith W J, Dunn M A. Medical defense against blistering chemical warfare agents[J]. *Arch Dermatol*, 1991, 127: 1207-1213.
- [18] Chiesa C, Pellegrini G, Panero A, De Luca T, Assumma M, Signore F, et al. Umbilical cord interleukin-6 levels are elevated in term neonates with perinatal asphyxia[J]. *Eur J Clin Invest*, 2003, 33: 352-358.
- [19] Cheong Y C, Shelton J B, Laird S M, Richmond M, Kudesia G, Li T C, et al. IL-1, IL-6 and TNF-alpha concentrations in the peritoneal fluid of women with pelvic adhesions[J]. *Hum Reprod*, 2002, 17: 69-75.
- [20] Arican O, Aral M, Sasmaz S, Ciragil P. Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity[J]. *Mediators Inflamm*, 2005, 2005: 273-279.
- [21] 华佳叶, 周旭红, 庞纓, 黄仁魏. 供受体血清白介素-2、肿瘤坏死因子- α 水平与急性移植植物抗宿主病的关系[J]. *南方医科大学学报*, 2010, 30: 831-833.

[本文编辑] 魏学丽, 邓晓群