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圣草酚调节IL-6/STAT3信号通路对支原体肺炎幼鼠肺组织的保护作用

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摘要 该文探究了圣草酚(ERD)调节白细胞介-6(IL-6)/信号转导和转录激活因子3(STAT3)信号通路对支原体肺炎(MP)幼鼠肺组织的保护作用。构建MP小鼠模型, 建模成功的小鼠随机分为Model组、ERD低剂量组、ERD中剂量组、ERD高剂量组、rIL-6(IL-6/STAT3信号通路激活剂)组, 每组10只小鼠。另外取10只正常小鼠为Con组, Con组与Model组给予等量的氯化钠溶液。测量小鼠肺湿重指数; 全自动血气分析仪检测小鼠动脉血氧分压和二氧化碳分压; HE染色和Masson染色观察小鼠肺组织的病理变化; TUNEL染色观察小鼠肺组织细胞凋亡情况; ELISA检测小鼠血清炎性相关因子表达情况; Western blot检测小鼠肺组织凋亡相关蛋白和IL-6/STAT3信号通路相关蛋白表达情况。实验结果显示, Model组小鼠肺组织病理损伤严重, 大量胶原纤维沉积。Model组肺湿重指数、二氧化碳分压、细胞凋亡率高于Con组, 肺组织Bax、Caspase-3、p-STAT3/STAT3表达水平高于Con组, 血清IL-8、TNF-α、IL-1β、IL-6水平高于Con组(P<0.05), 血氧分压低于Con组, 血清IL-10水平低于Con组, 肺组织Bcl-2表达水平低于Con组(P<0.05); ERD低剂量组、ERD中剂量组、ERD高剂量组小鼠肺组织病理损伤减轻, 胶原纤维沉积减少。ERD低剂量组、ERD中剂量组、ERD高剂量组肺湿重指数、二氧化碳分压、细胞凋亡率低于Model组, 肺组织Bax、Caspase-3、p-STAT3/STAT3表达水平低于Model组, 血清IL-8、TNF-α、IL-1β、IL-6水平低于Model组(P<0.05), 血氧分压高于Model组, 血清IL-10高于Model组, 肺组织Bcl-2表达水平高于Model组(P<0.05); rIL-6组小鼠肺组织病理损伤加重, 胶原纤维沉积增多。rIL-6组肺湿重指数、二氧化碳分压、细胞凋亡率低于ERD高剂量组, 肺组织Bax、Caspase-3、p-STAT3/STAT3表达水平高于ERD高剂量组, 血清IL-8、TNF-α、IL-1β、IL-6水平高于ERD高剂量组(P<0.05), 血氧分压低于ERD高剂量组, 血清IL-10低于ERD高剂量组, 肺组织Bcl-2表达水平低于ERD高剂量组(P<0.05)。综上所述, ERD通过抑制IL-6/STAT3信号通路, 降低炎症相关因子水平, 对MP幼鼠肺组织起到保护作用。

关键词 圣草酚; 白细胞介素6/信号转导和转录激活因子3; 支原体肺炎; 肺组织

The Protective Effect of Eriodictyol on Lung Tissue of Young Mice with Mycoplasma Pneumonia by Regulating IL-6/STAT3 Signaling Pathway

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Abstract This paper investigated the protective effect of ERD (Eriodictyol) on the lung tissue of young

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mice with MP (mycoplasma pneumonia) by regulating the IL-6 (interleukin-6)/STAT3 (signal transducer and activator of transcription 3) signaling pathway. An MP mouse model was constructed, and successfully modeled mice were stochastically assigned into Model group, low-dose ERD group, medium-dose ERD group, high-dose ERD group, and rIL-6 (IL-6/STAT3 signaling pathway activator) group, each had 10 mice. In addition, 10 normal mice were included as the Con group, and an equal amount of sodium chloride solution was given to both the Con group and the Model group. The wet weight index of mouse lungs was measured. Fully automatic blood gas analyzer was used to detect arterial oxygen partial pressure and carbon dioxide partial pressure in mice. HE staining and Masson staining were performed to observe the pathological changes in mouse lung tissue. TUNEL staining was performed to observe apoptosis of mouse lung tissue cells. ELISA was used to detect the inflammatory related factors in mouse serum. Western blot was performed to detect the apoptosis related proteins and IL-6/STAT3 signaling pathway related proteins in mouse lung tissue. The results showed that the pathological damage of lung tissue in the Model group was severe, with inflammatory cell infiltration and a large amount of collagen fiber deposition. The lung wet weight index, partial pressure of carbon dioxide, and apoptosis rate in the Model group were higher than those in the Con group. The expressions of Bax, Caspase-3, and p-STAT3/STAT3 in the lung tissue in the Model group were higher than those in the Con group, and the levels of serum IL-8, TNF-α, IL-1β, and IL-6 were higher than those in the Con group (P<0.05). In the Model group, the blood oxygen partial pressure was lower than that in the Con group, the serum IL-10 level was lower than that in the Con group, and the expression of Bcl-2 in the lung tissue was lower than that in the Con group (P<0.05). The low-dose, medium-dose, and high-dose ERD groups showed reduced pathological damage to lung tissue, decreased inflammatory cell infiltration and collagen fiber deposition. In the low-dose, medium-dose, and high-dose ERD groups, the lung wet weight index, carbon dioxide partial pressure, cell apoptosis rate were lower than those in the Model group. In the low-dose, medium-dose, and high-dose ERD groups, the expressions of Bax, Caspase-3, p-STAT3/STAT3 in lung tissue were lower than those in the Model group, and the levels of serum IL-8, TNF-α, IL-1β, IL-6 were lower than those in the Model group (P<0.05). In the low-dose, medium-dose, and high-dose ERD groups, the blood oxygen partial pressure was higher than that in the Model group, and the IL-10 level was higher than that in the Model group. The expression of Bcl-2 than that in lung tissue was higher than that in the Model group (P<0.05). The lung tissue pathological damage of mice in the rIL-6 group worsened, with increased inflammatory cell infiltration and collagen fiber deposition. The lung wet weight index, partial pressure of carbon dioxide, and apoptosis rate were lower than those in the ERD high-dose group. The expressions of Bax, Caspase-3 and p-STAT3/STAT3 in lung tissue were higher than those in the ERD high-dose group. The levels of serum IL-8, TNF-α, IL-1β, and IL-6 were higher than those in the high-dose ERD group (P<0.05). The blood oxygen partial pressure was lower than that in the ERD high-dose group, and the serum IL-10 was lower than that in the ERD high-dose group. The expression of Bcl-2 in lung tissue was lower than that in the ERD high-dose group (P<0.05). To sum up, ERD exerts a protective effect on the lung tissue of MP mice by inhibiting IL-6/STAT3 signaling pathway and suppressing inflammatory cell infiltration.

Keywords Eriodictyol; interleukin-6/signal transducer and activator of transcription 3; mycoplasma pneumonia; lung tissue

支原体肺炎(mycoplasma pneumonia, MP)是由肺炎支原体引起的一种呼吸道感染疾病, 常发生于儿童和青少年, 其在世界范围内发病率和死亡率都在逐年增加[1-2]。MP常见症状主要是发热、咳嗽, 严重者可能出现呼吸困难、胸痛等, 影响患者的健康, 对全球公共卫生造成重大的疾病负担[3]。目前MP的治疗是使用抗生素, 例如大环内酯类、四环素类药物等[4]。然而, 随着过量使用抗生素可能导致机体产

生耐药性, 不利于患者预后[5]。迫切需要研发新的治疗方法或者有效药物治疗MP。圣草酚(Eriodictyol, ERD)属于一种天然存在的黄酮类化合物, 广泛存在于多种植物, 如柠檬、薄荷、柑橘类水果以及某些草药中[6]。ERD具有多种生物学活性, 在抗氧化、抗炎、抗癌、神经保护等方面具有广泛的应用前景[7-8]。已有研究发现ERD能够保护小鼠免受耐甲氧西林金黄色葡萄球菌诱导的肺炎侵害[9]。猜测ERD可能对MP引起的肺损伤具有潜在保护作用。白细胞介素-6(interleukin-6, IL-6)是一种多效应性细胞因子, 参与免疫应答、炎症、骨代谢等过程[10]。信号转导和转录激活因子3(signal transducer and activator of transcription 3, STAT3)是STAT家族成员之一, 在炎症、心血管疾病、肿瘤进展等病理过程中起重要作用[11]。IL-6/STAT3信号通路在免疫调节、炎症反应、细胞增殖、分化和凋亡过程中发挥关键作用。有研究表明IL-6/STAT3信号通路的激活导致肺功能下降, 促进MP的发生和发展[12]。猜测IL-6/STAT3信号通路可能参与减轻MP诱导的肺组织损伤。目前尚不清楚ERD是否能够通过调控IL-6/STAT3信号通路对MP小鼠肺组织起到保护作用。因此, 本研究通过构建MP小鼠模型, 旨在研究ERD对MP小鼠肺组织的影响以及可能的机制, 为MP的治疗提供新见解。

1 材料与方法

1.1 实验动物

本研究选用购自昭衍(苏州)新药研究中心的SPF级BALB/c小鼠[4~5周龄, 体质量(20±2) g], 生产许可证号: SCXK(苏)2024-0008。小鼠饲养于温度(23±2) ℃、相对湿度50%、明暗交替各12 h的SPF级实验动物房, 给予充足的饲料和纯净水, 自由活动。动物实验均经过保定市第二中心医院伦理委员会的批准(伦理批号: 伦2024-0113)。

1.2 主要试剂与仪器

ERD(货号: T6S0232)购自美国Target Molecule Corp公司; IL-6/STAT3信号通路激活剂rIL-6(货号: 90146ES05)购自翌圣生物科技(上海)股份有限公司; 肺炎支原体(货号: 0503L)购自中国工业微生物菌种保藏管理中心; HE染色试剂盒、Masson染色试剂盒(货号: ZY61873FA、ZY660314)购自上海泽叶生物科技有限公司; TUNEL凋亡检测试剂盒(货号: A111-01)购自南京诺唯赞生物科技股份有限公司; 小鼠IL-8 ELISA试剂盒(货号: EM1592)、小鼠TNF-α ELISA试剂盒(货号: EM0183)、小鼠IL-1β ELISA试剂盒(货号: EM0109)和小鼠IL-10 ELISA试剂盒(货号: EM0100)购自武汉菲恩生物科技有限公司; SDS-PAGE凝胶配制试剂盒、RIPA裂解液、BCA蛋白浓度测定试剂盒(货号: SW108、SW206、SW101)购自赛文创新(北京)生物科技有限公司; 一抗Bax、Caspase-3、Bcl-2、IL-6、p-STAT3、STAT3、GAPDH、山羊抗兔IgG(H+L)(货号: AF0120、AF6311、AF6139、DF6087、AF3293、AF6294、AF0911、S0001)购自江苏亲科生物研究中心有限公司; 全自动血气分析仪(型号: Derry D15 VET)购自深圳市前沿新技术有限公司; 倒置荧光显微镜(型号: BDS400)购自重庆奥特光学仪器有限公司; 多功能酶标仪(型号: EnVision)购自浙江蓝箭仪器有限公司; 凝胶成像系统(型号: JY04S-3C)购自北京君意东方电泳设备有限公司。

1.3 实验方法

1.3.1 MP小鼠模型构建及分组处理 按照吴文娟等[13]的文献方法构建MP小鼠模型。将低温冻存的肺炎支原体菌种(中国工业微生物菌种保藏管理中心)进行复苏, 然后在恒温培养箱(37 ℃、5% CO_2)进行培养, 并通过稀释, 配制成浓度为3×10^7 CCU/mL的肺炎支原体悬液, 用于后续小鼠造模。小鼠使用水合氯醛麻醉, 然后通过小鼠鼻部接种20 μL浓度为1×10^7 CCU/mL的肺炎支原体悬液, 每天接种1次, 持续干预3天。小鼠出现呼吸频率加快、寒颤、活动减少且支原体核酸检测阳性, 提示MP小鼠模型构建成功。

将建模成功的小鼠随机分成Model组, ERD低剂量组、ERD中剂量组、ERD高剂量组(分别腹腔注射20、40、80 mg/kg ERD[14]), rIL-6组(腹腔注射80 mg/kg ERD和腹腔注射0.1 mg/kg的rIL-6[15]), 每组各10只。另取10只正常小鼠为Con组, Con组与Model组腹腔注射等量氯化钠溶液, 持续7天。

1.3.2 样品收集 末次给药结束后, 先通过精准天平对各组小鼠进行称重并记录, 然后将各组小鼠进行眼球取血, 4 ℃静置1.5 h, 再将其置于4 ℃预冷离心机, 1 500 r/min、4 ℃离心20 min, 收集血清至离心管, 保存于–20 ℃冰箱备用。脱颈处死小鼠, 收集各组小鼠肺组织, 通过天平测量肺湿重并记录, 最后保存于–80 ℃冰箱, 用于后续实验。

1.3.3 小鼠肺湿重指数测定 利用1.3.2记录的各

组小鼠体质量和肺湿重计算肺湿重指数。

1.3.4 动脉血氧分压和二氧化碳分压检测 取–20 ℃保存的血清, 然后通过全自动血气分析仪检测各组小鼠血清动脉血氧分压和二氧化碳分压。

1.3.5 HE染色和Masson染色观察小鼠肺组织的病理变化 取–80 °C保存的部分肺组织, 快速解冻后, 置于多聚甲醛中4 °C固定过夜, 然后脱水、石蜡包埋, 石蜡切片机制成切片。将切片进行脱蜡处理后, 使用HE染色试剂盒和Masson染色试剂盒分别处理切片, PBS清洗切片, 封片后在显微镜下观察小鼠的肺组织的病理变化和胶原纤维变化。

1.3.6 TUNEL染色观察小鼠肺组织细胞凋亡 将切片进行脱蜡处理后, 使用TUNEL凋亡检测试剂盒处理, 显微镜下观察各组小鼠肺组织细胞的凋亡情况, 绿色荧光代表凋亡细胞。

1.3.7 ELISA检测小鼠血清炎性相关因子表达 取–20 °C保存的血清, 快速解冻后, 根据ELISA检测试剂盒说明书, 对标准品和待测血清样本进行稀释。将稀释后的标准品和待测血清样本加入96孔板中, 加入生物素标记抗体孵育1 h, 然后加入辣根过氧化物酶标记的二抗37 °C孵育30 min, 最后加入显色底物, 避光孵育20 min后, 加入终止液终止反应, 利用多功能酶标仪检测各组小鼠血清在450 nm处的D值, 根据数据绘制标准曲线, 然后计算出各组小鼠血清中IL-8、TNF-α、IL-1β、IL-10的浓度。

1.3.8 Western blot检测小鼠肺组织中凋亡和通路相关蛋白表达情况 将小鼠肺组织快速解冻后, 加入含蛋白酶抑制剂的蛋白裂解液进行匀浆, 12 000 ×g、4 °C离心15 min提取总蛋白, 然后进行蛋白定量, 计算出相应的上样量, 蛋白变性后, 进行电泳分离蛋白, 将蛋白转至PVDF膜, 蛋白快速封闭液室温封闭30 min, TBST清洗蛋白条带3次, 将蛋白条带与对应的一抗Bax、Caspase-3、Bcl-2、IL-6、p-STAT31、STAT3(稀释比例1:1 000) 4 °C孵育过夜, TBST清洗蛋白条带, 与对应种属二抗(1:2 000)室温孵育2 h, 滴加化学发光显色液, 通过凝胶成像系统拍照, ImageJ图像软件分析蛋白条带, 计算不同蛋白表达量。

1.4 统计学分析及处理

数据分析处理主要利用SPSS 26.0软件。不同计量资料数据结果用均数±标准差($\bar{x}\pm s$)表示。多组间比较采用单因素方差分析, 两组间比较采用SNK-q检验。差异有统计学意义被设定为P<0.05。

2 结果

2.1 ERD对MP小鼠肺湿重指数、动脉血氧分压和二氧化碳分压的影响

Model组小鼠肺湿重指数、二氧化碳分压高于Con组(P<0.05), 血氧分压低于Con组(P<0.05); ERD低剂量组、ERD中剂量组、ERD高剂量组小鼠肺湿重指数、二氧化碳分压显著低于Model组(P<0.05), 血氧分压显著高于Model组(P<0.05); rIL-6组小鼠肺湿重指数、二氧化碳分压显著高于ERD高剂量组(P<0.05), 血氧分压显著低于ERD高剂量组(P<0.05)。见表1。

2.2 ERD对MP小鼠肺组织的病理变化的影响

Model组与Con组相比, 小鼠肺组织病理损伤严

表1 各组小鼠肺湿重指数、动脉血氧分压和二氧化碳分压比较

Table 1 Comparison of lung wet weight index, arterial oxygen partial pressure and carbon dioxide partial pressure among different groups of mice

组别 Groups	肺湿重指数/% Lung wet weight index /%	动脉血氧分压/mmHg Arterial oxygen partial pressure /mmHg	二氧化碳分压/mmHg Partial pressure of carbon dioxide /mmHg
Con group	0.45±0.05	99.38±10.69	42.58±4.39
Model group	1.05±0.11^{a}	52.49±6.28^{a}	91.37±9.53^{a}
ERD low-dose group	0.89±0.09^{b}	63.15±6.44^{b}	80.27±8.16^{b}
ERD medium-dose group	0.75±0.15^{bc}	78.49±7.86^{bc}	66.81±6.87^{bc}
ERD high-dose group	0.56±0.06^{bcd}	92.25±11.93^{bcd}	51.97±5.24^{bcd}
rIL-6 group	0.97±0.12^{e}	59.74±6.57^{e}	86.45±8.75^{e}

$\bar{x}\pm s$, n=10; ^{a}P<0.05, 与Con组比较; ^{b}P<0.05, 与Model组比较; ^{c}P<0.05, 与ERD低剂量组比较; ^{d}P<0.05, 与ERD中剂量组比较; ^{e}P<0.05, 与ERD高剂量组比较。

$\bar{x}\pm s$, n=10; ^{a}P<0.05 compared with the Con group; ^{b}P<0.05 compared with the Model group; ^{c}P<0.05 compared with the ERD low-dose group; ^{d}P<0.05 compared with the ERD medium-dose group; ^{e}P<0.05 compared with the ERD high-dose group.

重，炎性细胞浸润，大量胶原纤维沉积；ERD低剂量组、ERD中剂量组、ERD高剂量组与Model组相比，小鼠肺组织病理损伤减轻，炎性细胞浸润和胶原纤维沉积减少；rIL-6组与ERD高剂量组相比，小鼠肺组织病理损伤加重，炎性细胞浸润和胶原纤维沉积增多。见图1和图2。

2.3 ERD对MP小鼠肺组织细胞凋亡的影响

Model组小鼠肺组织细胞凋亡率，Bax、Caspase-3表达水平高于Con组（$P<0.05$），Bcl-2表达水平低于Con组（$P<0.05$）；ERD低剂量组、ERD中剂量组、ERD高剂量组小鼠肺组织细胞凋亡率，Bax、Caspase-3表达水平显著低于Model组（$P<0.05$），Bcl-2表达水平显著高于Model组（$P<0.05$）；rIL-6组小鼠肺组织细胞凋亡率，Bax、Caspase-3表达水平显著高于ERD高剂量组（$P<0.05$），Bcl-2表达水平显著高于ERD高剂量组（$P<0.05$）。见图3、图4和表2。

2.4 ERD对MP小鼠血清炎性相关因子表达水平的影响

Model组小鼠血清IL-8、TNF-α、IL-1β表达水平高于Con组（$P<0.05$），IL-10表达水平低于Con组

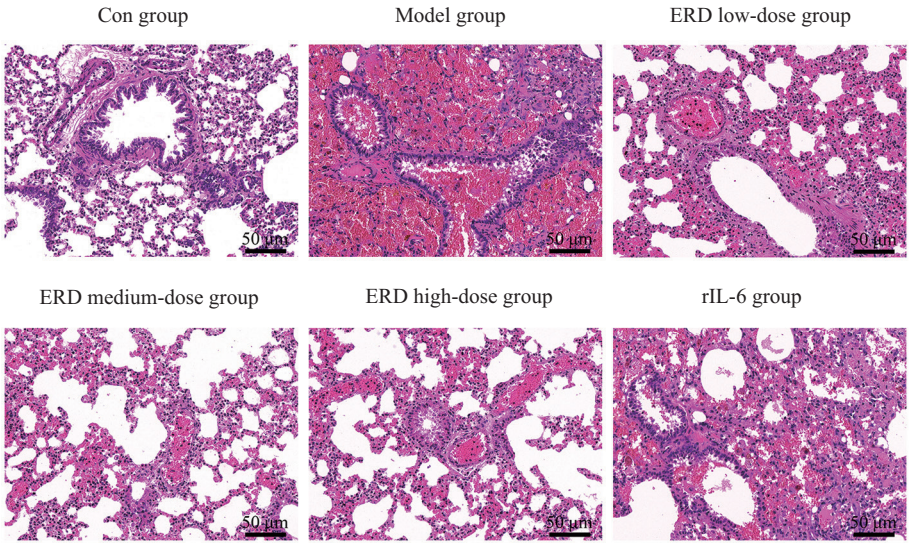


图1 HE染色观察各组小鼠肺组织病理变化

Fig.1 HE staining shows the pathological changes of lung tissues in each group of mice

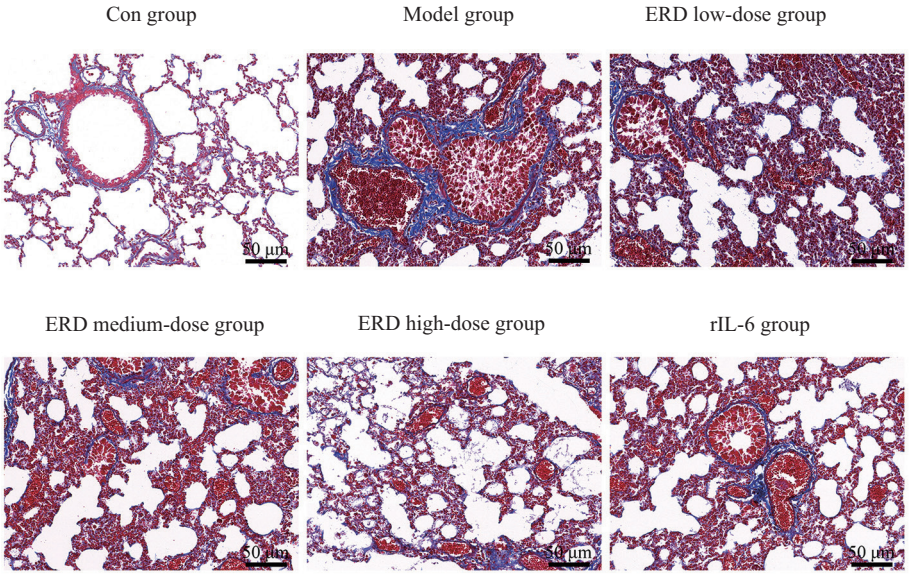


图2 Masson染色观察各组小鼠肺组织胶原纤维变化

Fig.2 Observation of collagen fiber changes in lung tissues of mice in each group by Masson staining

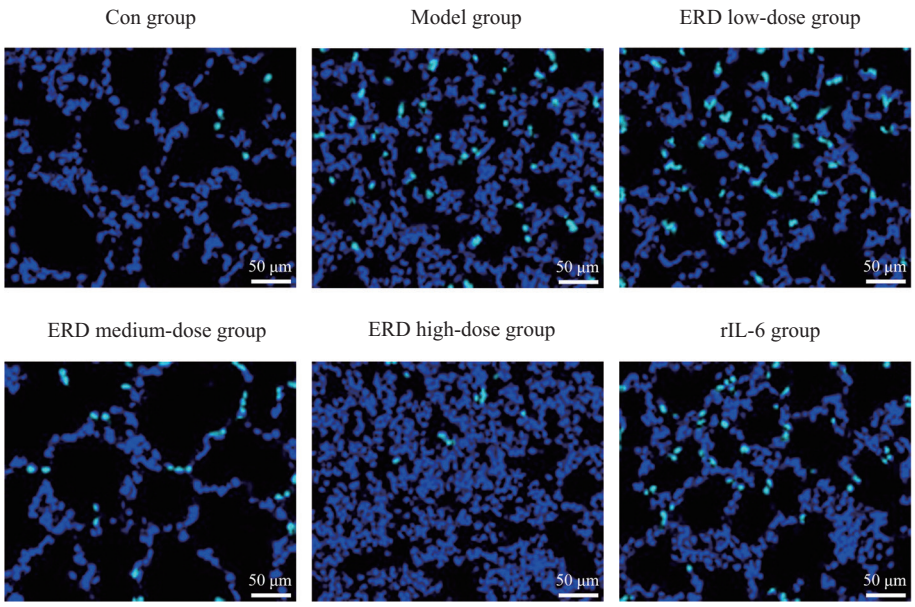
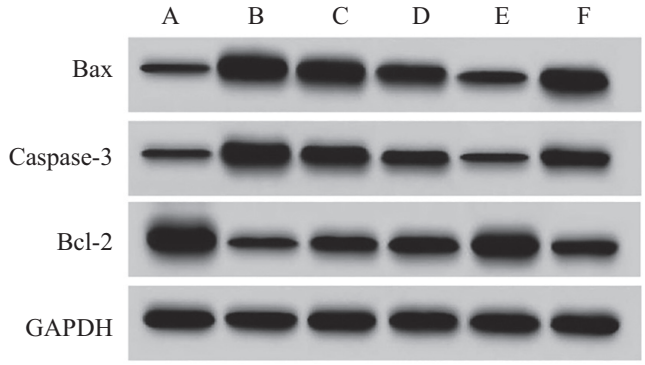


图3 TUNEL染色观察小鼠肺组织细胞凋亡变化

Fig.3 Observation of apoptosis changes in mouse lung tissue by TUNEL staining



A: Con组; B: Model组; C: ERD低剂量组; D: ERD中剂量组; E: ERD高剂量组; F: rIL-6组。

A: Con group; B: Model; C: ERD low-dose group; D: ERD medium-dose group; E: ERD high-dose group; F: rIL-6 group.

图4 Western blot检测小鼠肺组织的凋亡相关蛋白表达情况

Fig.4 Western blot analysis of the expression levels of apoptosis-related proteins in mouse lung tissue

表2 各组小鼠肺组织凋亡相关蛋白表达比较

Table 2 Comparison of expression levels of apoptosis-related proteins in lung tissues of each group of mice

组别 Groups	凋亡率/% Apoptosis rate /%	Bax	Caspase-3	Bcl-2
Con group	2.35±0.25	0.58±0.06	0.39±0.04	1.27±0.15
Model group	31.48±3.76^{a}	1.15±0.12^{a}	1.06±0.13^{a}	0.61±0.07^{a}
ERD low-dose group	25.61±2.68^{b}	1.03±0.11^{b}	0.91±0.12^{b}	0.78±0.09^{b}
ERD medium-dose group	16.93±1.82^{bc}	0.86±0.09^{bc}	0.74±0.08^{bc}	0.99±0.11^{bc}
ERD high-dose group	5.49±0.63^{bcd}	0.67±0.07^{bcd}	0.55±0.06^{bcd}	1.13±0.12^{bcd}
rIL-6 group	27.58±2.84^{e}	1.07±0.13^{e}	0.98±0.11^{e}	0.72±0.08^{e}

$\bar{x}\pm s$, n=10; ^{a}P<0.05, 与Con组比较; ^{b}P<0.05, 与Model组比较; ^{c}P<0.05, 与ERD低剂量组比较; ^{d}P<0.05, 与ERD中剂量组比较; ^{e}P<0.05, 与ERD高剂量组比较。

$\bar{x}\pm s$, n=10; ^{a}P<0.05 compared with the Con group; ^{b}P<0.05 compared with the Model group; ^{c}P<0.05 compared with the ERD low-dose group; ^{d}P<0.05 compared with the ERD medium-dose group; ^{e}P<0.05 compared with the ERD high-dose group.

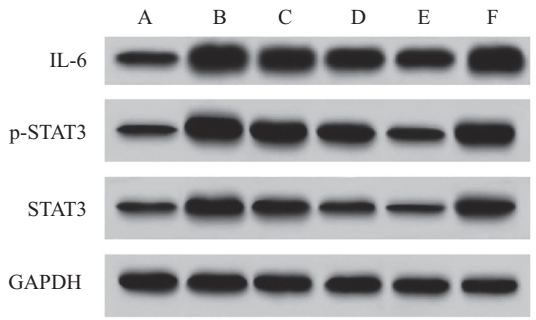
表3 各组小鼠血清炎性相关因子表达比较

Table 3 Comparison of expression levels of inflammatory-related factors in serum of each group of mice

组别 Groups	IL-8 /ng·L^{-1}	TNF-α /ng·L^{-1}	IL-1β /ng·L^{-1}	IL-10 /pg·mL^{-1}
Con group	286.58±31.92	310.79±36.41	55.97±6.28	298.75±31.69
Model group	531.76±58.43^{a}	628.17±69.55^{a}	124.86±15.03^{a}	86.91±9.27^{a}
ERD low-dose group	475.21±51.67^{b}	560.83±61.39^{b}	103.45±11.52^{b}	115.79±12.82^{b}
ERD medium-dose group	407.63±43.84^{bc}	474.53±53.82^{bc}	82.62±8.27^{bc}	183.53±19.43^{bc}
ERD high-dose group	323.94±35.78^{bcd}	385.96±42.67^{bcd}	69.73±7.65^{bcd}	257.36±28.71^{bcd}
rIL-6 group	513.87±54.21^{e}	589.41±64.83^{e}	116.24±12.96^{e}	96.25±10.45^{e}

$\bar{x}\pm s$, n=10; ^{a}P<0.05, 与Con组比较; ^{b}P<0.05, 与Model组比较; ^{c}P<0.05, 与ERD低剂量组比较; ^{d}P<0.05, 与ERD中剂量组比较; ^{e}P<0.05, 与ERD高剂量组比较。

$\bar{x}\pm s$, n=10; ^{a}P<0.05 compared with the Con group; ^{b}P<0.05 compared with the Model group; ^{c}P<0.05 compared with the ERD low-dose group; ^{d}P<0.05 compared with the ERD medium-dose group; ^{e}P<0.05 compared with the ERD high-dose group.



A: Con组; B: Model组; C: ERD低剂量组; D: ERD中剂量组; E: ERD高剂量组; F: rIL-6组。

A: Con group; B: Model; C: ERD low-dose group; D: ERD medium-dose group; E: ERD high-dose group; F: rIL-6 group.

图5 Western blot检测小鼠肺组织IL-6/STAT3信号通路相关蛋白表达情况

Fig.5 Western blot analysis of the expression levels of proteins related to the IL-6/STAT3 signaling pathway in mouse lung tissue

(P<0.05); ERD低剂量组、ERD中剂量组、ERD高剂量组小鼠血清IL-8、TNF-α、IL-1β表达水平显著低于Model组(P<0.05), IL-10表达水平显著高于Model组(P<0.05); rIL-6组小鼠血清IL-8、TNF-α、IL-1β表达水平显著高于ERD高剂量组(P<0.05), IL-10表达水平显著低于ERD高剂量组(P<0.05)。见表3。

2.5 ERD对MP小鼠肺组织IL-6/STAT3信号通路相关蛋白表达的影响

Model组小鼠肺组织IL-6、p-STAT3/STAT3表达水平高于Con组(P<0.05); ERD低剂量组、ERD中剂量组、ERD高剂量组小鼠肺组织IL-6、p-STAT3/STAT3表达水平显著低于Model组(P<0.05); rIL-6组小鼠肺组织IL-6、p-STAT3/STAT3表达水平显著高于ERD高剂量组(P<0.05)。见图5和表4。

3 讨论

MP是引起儿童和青少年社区获得性肺炎的主要原因[16]。大环内酯类抗生素已经被广泛用于治疗MP, 并取得良好的临床效果。然而, 近年来, 部分患者虽然使用抗生素治疗, 但仍然出现持续性发热现象, 临床症状恶化, 甚至引发严重的肺部并发症[17]。因此, 需要进一步探索MP发生和发展机制, 并开发新型药物或替代疗法, 改善MP患者的治疗结局。本研究采用小鼠鼻部滴入肺炎支原体菌种溶液构建MP小鼠模型发现, Model组小鼠肺湿重指数增加, 血氧分压降低, 二氧化碳分压升高, 肺组织病理改变严重, 胶原纤维沉积和细胞凋亡增加, 血清炎性因子IL-8、TNF-α、IL-1β高表达, IL-10低表达, 提示肺组织大量炎性细胞的浸润, 促进病理损伤和细胞凋亡, 加重MP病情。

已有研究发现, STAT3是IL-6的下游靶点, IL-6通过磷酸化STAT3, 激活IL-6/STAT3信号通路, 进而参与炎症反应过程[18]。在MP进展过程中, 由于肺组织在肺炎支原体诱导下产生过度的炎症反应对肺组

表4 各组小鼠肺组织IL-6/STAT3信号通路相关蛋白表达比较

Table 4 Comparison of IL-6/STAT3 signaling pathway-related protein expressions in lung tissues of each group of mice

组别 Groups	IL-6/GAPDH	p-STAT3/STAT3
Con group	0.68±0.07	0.26±0.03
Model group	1.45±0.15[a]	0.99±0.11[a]
ERD low-dose group	1.29±0.14[b]	0.85±0.09[b]
ERD medium-dose group	1.06±0.12[bc]	0.62±0.07[bc]
ERD high-dose group	0.79±0.09[bcd]	0.38±0.05[bcd]
rIL-6 group	1.37±0.14[e]	0.91±0.12[e]

$\bar{x}\pm s$, n=10; [a]P<0.05, 与Con组比较; [b]P<0.05, 与Model组比较; [c]P<0.05, 与ERD低剂量组比较; [d]P<0.05, 与ERD中剂量组比较; [e]P<0.05, 与ERD高剂量组比较。

$\bar{x}\pm s$, n=10; [a]P<0.05 compared with the Con group; [b]P<0.05 compared with the Model group; [c]P<0.05 compared with the ERD low-dose group; [d]P<0.05 compared with the ERD medium-dose group; [e]P<0.05 compared with the ERD high-dose group.

织造成损伤, 表明IL-6/STAT3信号通路可能成为治疗MP的潜在靶点。CHEN等[19]研究显示, 补骨脂异黄酮通过抑制IL-6/STAT3信号通路, 抑制炎性细胞浸润和炎性细胞因子表达, 减轻脂多糖诱导的急性肺损伤。KAMEL等[20]研究发现, 阿伐美拉汀通过抑制IL-6/STAT3信号通路, 抑制氧化应激和炎症过程, 对甲氨蝶呤诱导的肺损伤具有保护作用。曹文婷等[21]研究表明, 白芍总苷抑制IL-6/STAT3信号通路的活化, 有效减轻脂多糖诱导的急性肺炎小鼠炎症反应, 改善肺组织的病理损伤。以上说明抑制IL-6/STAT3信号通路对肺组织损伤具有保护作用。本研结果发现, 与Con组比较, Model组小鼠肺组织IL-6、p-STAT3/STAT3的蛋白表达水平升高, 提示IL-6/STAT3信号通路的激活可能是导致MP小鼠肺组织病理损伤加重的原因。

ERD是一种从草药中提取得到的黄酮类产物, 具有多种治疗特性, 包括抗氧化、抗炎等[22]。最近已有研究发现, ERD能够减轻肺损伤, 对肺组织起到保护作用。WANG等[9]研究显示, ERD抑制分选酶A表达, 减弱耐甲氧西林金黄色葡萄球菌的毒力, 防止耐药性的产生, 在金黄色葡萄球菌诱导的小鼠肺炎模型中展现出良好的治疗效果。WANG等[14]研究表明, ERD通过抑制COX-2/NLRP3/NF-κB信号通路的转导, 抑制炎症因子TNF-α、IL-6、IL-1β、前列腺素E2的表达, 改善肺部病理变化, 对脂多糖诱导的急性肺损伤具有保护作用。以上均显示ERD能够抑制炎症反应的发生, 有助于肺部损伤恢复。本研究结果发现, 不同剂量的ERD处理小鼠能够显著降低IL-6、p-STAT3/STAT3的蛋白表达水平, 抑制IL-6/STAT3信号通路, 降低肺湿重指数, 恢复血氧分压和二氧化碳分压, 减轻肺组织病理改变和细胞凋亡, 抑制炎性相关因子IL-8、TNF-α、IL-1β表达, 促进IL-10表达, 改善MP小鼠肺组织损伤。而联合使用rIL-6时, 能够逆转ERD对MP小鼠肺组织的保护作用, 提示ERD可能通过抑制IL-6/STAT3信号通路, 抑制炎症反应, 改善MP小鼠肺组织的病理变化, 对肺组织具有保护作用。

综上所述, ERD对MP小鼠肺组织的保护效应, 其作用机制可能是通过抑制IL-6/STAT3信号通路来实现的, 这一发现为开发新型药物治疗MP提供了新的研究方向。由于MP发病机制的复杂性, 仍然需要仍然需要继续深入探索ERD治疗MP的其他具体分子机制, 为临床提供更加全面的参考依据。

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