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# 基于 Toll 样受体 4/核因子 $\kappa$ B 信号通路探究苦参碱对类风湿关节炎风湿热痹证的治疗作用及机制

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**摘要 目的:**基于 Toll 样受体 4(Toll-like receptor 4, TLR4)/核因子  $\kappa$ B(nuclear factor- $\kappa$ B, NF- $\kappa$ B) 信号通路探究苦参碱对类风湿关节炎(rheumatoid arthritis, RA)风湿热痹证的治疗作用及机制。**方法:**将 60 只 8 周龄 SPF 级雄性 SD 大鼠随机分为正常组、模型组、甲氨蝶呤组及苦参碱低、中、高剂量组, 每组 10 只。除正常组外, 其余 5 组均采用牛Ⅱ型胶原诱导联合人工气候箱干预建立 RA 风湿热痹证模型。甲氨蝶呤组大鼠按照  $1.0 \text{ mg} \cdot \text{kg}^{-1}$  以甲氨蝶呤灌胃, 苦参碱低、中、高剂量组大鼠分别按照  $30 \text{ mg} \cdot \text{kg}^{-1}$ 、 $60 \text{ mg} \cdot \text{kg}^{-1}$ 、 $120 \text{ mg} \cdot \text{kg}^{-1}$  以苦参碱灌胃, 正常组和模型组大鼠则以等体积蒸馏水灌胃。药物干预均每周 1 次, 共干预 4 次。观察大鼠的一般情况, 测定足趾肿胀度、关节炎指数, 以 ELISA 法检测血清肿瘤坏死因子- $\alpha$ (tumor necrosis factor- $\alpha$ , TNF- $\alpha$ )、白细胞介素- $1\beta$ (interleukin- $1\beta$ , IL- $1\beta$ ) 含量, HE 染色观察膝关节滑膜组织病理学改变, 以实时定量 PCR 法检测膝关节滑膜组织 Bax mRNA、Bcl-2 mRNA、TLR4 mRNA、NF- $\kappa$ B p65 mRNA 表达量, 以 Western Blot 法检测膝关节滑膜组织 Cleaved caspase-3 蛋白、TLR4 蛋白、NF- $\kappa$ B p65 蛋白、磷酸化核因子  $\kappa$ B p65(phosphorylated nuclear factor- $\kappa$ B p65, p-NF- $\kappa$ B p65) 蛋白表达量。**结果:**①大鼠一般情况。正常组大鼠毛发顺滑有光泽, 饮食、饮水正常, 足趾无肿胀; 造模后模型组、甲氨蝶呤组及苦参碱低、中、高剂量组大鼠均出现毛发干燥无光泽, 摄水量增加, 易激惹、攻击性强, 足趾肿胀、红热、蜷缩、僵硬等表现; 与模型组相比, 药物干预后甲氨蝶呤组及苦参碱低、中、高剂量组大鼠毛发干燥无光泽、足趾肿胀等情况有所改善。②足趾肿胀度。6 组大鼠的足趾肿胀度比较, 差异有统计学意义( $0.08 \pm 0.01$ ,  $0.51 \pm 0.07$ ,  $0.24 \pm 0.04$ ,  $0.44 \pm 0.06$ ,  $0.37 \pm 0.04$ ,  $0.28 \pm 0.06$ ,  $F = 118.983$ ,  $P = 0.000$ )。模型组大鼠的足趾肿胀度高于其余 5 组( $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ), 苦参碱低剂量组大鼠的足趾肿胀度高于甲氨蝶呤组和苦参碱中、高剂量组( $P = 0.007$ ;  $P = 0.000$ ;  $P = 0.000$ ), 苦参碱中剂量组大鼠的足趾肿胀度高于甲氨蝶呤组和苦参碱高剂量组( $P = 0.001$ ;  $P = 0.000$ ), 甲氨蝶呤组和苦参碱高剂量组大鼠足趾肿胀度的差异无统计学意义( $P = 0.096$ )。③关节炎指数。正常组大鼠足部未见异常, 关节炎指数为 0 分; 其余 5 组大鼠关节炎指数比较, 差异有统计学意义[( $7.02 \pm 0.24$ )分, ( $4.36 \pm 0.12$ )分, ( $6.32 \pm 0.16$ )分, ( $5.58 \pm 0.20$ )分, ( $4.48 \pm 0.14$ )分,  $F = 422.684$ ,  $P = 0.000$ ]。模型组大鼠的关节炎指数高于甲氨蝶呤组和苦参碱低、中、高剂量组( $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ), 苦参碱低剂量组大鼠的关节炎指数高于甲氨蝶呤组和苦参碱中、高剂量组( $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ), 苦参碱中剂量组大鼠的关节炎指数高于甲氨蝶呤组和苦参碱高剂量组( $P = 0.000$ ;  $P = 0.000$ ), 甲氨蝶呤组和苦参碱高剂量组大鼠关节炎指数的差异无统计学意义( $P = 0.054$ )。④血清 TNF- $\alpha$ 、IL- $1\beta$  含量。6 组大鼠的血清 TNF- $\alpha$ 、IL- $1\beta$  含量比较, 组间差异均有统计学意义[TNF- $\alpha$ : ( $48.09 \pm 4.88$ )  $\text{pg} \cdot \text{mL}^{-1}$ , ( $351.49 \pm 32.39$ )  $\text{pg} \cdot \text{mL}^{-1}$ , ( $143.05 \pm 10.02$ )  $\text{pg} \cdot \text{mL}^{-1}$ , ( $281.51 \pm 26.50$ )  $\text{pg} \cdot \text{mL}^{-1}$ , ( $207.63 \pm 17.00$ )  $\text{pg} \cdot \text{mL}^{-1}$ , ( $154.40 \pm 14.23$ )  $\text{pg} \cdot \text{mL}^{-1}$ ,  $F = 311.253$ ,  $P = 0.000$ ; IL- $1\beta$ : ( $30.71 \pm 4.60$ )  $\text{pg} \cdot \text{mL}^{-1}$ , ( $258.14 \pm 20.85$ )  $\text{pg} \cdot \text{mL}^{-1}$ , ( $105.27 \pm 10.38$ )  $\text{pg} \cdot \text{mL}^{-1}$ , ( $201.57 \pm 16.51$ )  $\text{pg} \cdot \text{mL}^{-1}$ , ( $158.97 \pm 16.18$ )  $\text{pg} \cdot \text{mL}^{-1}$ , ( $114.37 \pm 10.48$ )  $\text{pg} \cdot \text{mL}^{-1}$ ,  $F = 337.119$ ,  $P = 0.000$ ]。模型组大鼠的血清 TNF- $\alpha$ 、IL- $1\beta$  含量均高于其余 5 组( $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ), 苦参碱低剂量组大鼠的血清 TNF- $\alpha$ 、IL- $1\beta$  含量均高于甲氨蝶呤组和苦参碱中、高剂量组( $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ), 苦参碱中剂量组大鼠的血清 TNF- $\alpha$ 、IL- $1\beta$  含量均高于甲氨蝶呤组和苦参碱高剂量组( $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ), 甲氨蝶呤组和苦参碱高剂量组大鼠血清 TNF- $\alpha$ 、IL- $1\beta$  含量的差异均无统计学意义( $P = 0.054$ ;  $P = 0.067$ )。⑤膝关节滑膜组织病理学观察结果。HE 染色结果显示, 正常组大鼠膝关节滑膜组织结构完整, 细胞排列整齐, 无炎症细胞浸润; 与正常组相比, 模型组大鼠滑膜组织增生明显, 有大量炎症细胞浸润, 滑膜细胞排列紊乱, 边界模糊不清; 与模型组相比, 甲氨蝶呤组和苦参碱低、中、高剂量组滑膜组织增生程度减轻, 滑膜细胞排列较整齐, 炎症细胞浸润情况均得到不同程度改善。⑥膝关节滑膜组织 Bax mRNA、Bcl-2 mRNA、TLR4 mRNA、NF- $\kappa$ B p65 mRNA 表达量。6 组大鼠膝关节滑膜组织 Bax mRNA、Bcl-2 mRNA、TLR4 mRNA、NF- $\kappa$ B p65 mRNA 表达量比较, 组间差异均有统计学意义(Bax mRNA:  $0.80 \pm 0.07$ ,  $0.40 \pm 0.03$ ,  $0.72 \pm 0.08$ ,  $0.53 \pm 0.05$ ,  $0.63 \pm 0.05$ ,  $0.71 \pm$

0.06,  $F = 69.870$ ,  $P = 0.000$ ; Bcl-2 mRNA:  $0.19 \pm 0.02$ ,  $0.78 \pm 0.06$ ,  $0.33 \pm 0.03$ ,  $0.67 \pm 0.05$ ,  $0.54 \pm 0.06$ ,  $0.36 \pm 0.04$ ,  $F = 258.197$ ,  $P = 0.000$ ; TLR4 mRNA:  $0.13 \pm 0.01$ ,  $0.61 \pm 0.07$ ,  $0.25 \pm 0.02$ ,  $0.54 \pm 0.05$ ,  $0.45 \pm 0.04$ ,  $0.27 \pm 0.03$ ,  $F = 206.811$ ,  $P = 0.000$ ; NF- $\kappa$ B p65 mRNA:  $0.17 \pm 0.01$ ,  $0.56 \pm 0.04$ ,  $0.26 \pm 0.02$ ,  $0.46 \pm 0.04$ ,  $0.34 \pm 0.04$ ,  $0.28 \pm 0.03$ ,  $F = 220.358$ ,  $P = 0.000$ 。模型组大鼠的膝关节滑膜组织 Bax mRNA 表达量低于其余 5 组 ( $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ) , Bcl-2 mRNA、TLR4 mRNA、NF- $\kappa$ B p65 mRNA 表达量均高于其余 5 组 ( $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.019$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ) ; 苦参碱低剂量组大鼠的膝关节滑膜组织 Bax mRNA 表达量低于甲氨蝶呤组和苦参碱中、高剂量组 ( $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ) , Bcl-2 mRNA、TLR4 mRNA、NF- $\kappa$ B p65 mRNA 表达量均高于甲氨蝶呤组及苦参碱中、高剂量组 ( $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ) ; 苦参碱中剂量组大鼠的膝关节滑膜组织 Bax mRNA 表达量低于甲氨蝶呤组和苦参碱高剂量组 ( $P = 0.000$ ;  $P = 0.000$ ) , Bcl-2 mRNA、TLR4 mRNA、NF- $\kappa$ B p65 mRNA 表达量均高于甲氨蝶呤组和苦参碱高剂量组 ( $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.001$ ) ; 甲氨蝶呤组和苦参碱高剂量组大鼠膝关节滑膜组织 Bax mRNA、Bcl-2 mRNA、TLR4 mRNA、NF- $\kappa$ B p65 mRNA 表达量的组间差异均无统计学意义 ( $P = 0.755$ ,  $P = 0.074$ ,  $P = 0.096$ ,  $P = 0.096$ )。⑦膝关节滑膜组织 Cleaved caspase-3 蛋白、TLR4 蛋白、NF- $\kappa$ B p65 蛋白、p-NF- $\kappa$ B p65 蛋白表达量。6 组大鼠的 Cleaved caspase-3 蛋白、TLR4 蛋白表达量及 p-NF- $\kappa$ B p65/NF- $\kappa$ B p65 蛋白表达量比值比较, 组间差异均有统计学意义 (Cleaved caspase-3 蛋白:  $0.74 \pm 0.06$ ,  $0.32 \pm 0.03$ ,  $0.62 \pm 0.05$ ,  $0.39 \pm 0.04$ ,  $0.47 \pm 0.05$ ,  $0.58 \pm 0.05$ ,  $F = 127.351$ ,  $P = 0.001$ ; TLR4 蛋白:  $0.17 \pm 0.02$ ,  $0.67 \pm 0.06$ ,  $0.25 \pm 0.03$ ,  $0.43 \pm 0.05$ ,  $0.35 \pm 0.03$ ,  $0.27 \pm 0.02$ ,  $F = 216.610$ ,  $P = 0.001$ ; p-NF- $\kappa$ B p65/NF- $\kappa$ B p65 蛋白表达量比值:  $0.24 \pm 0.02$ ,  $0.64 \pm 0.07$ ,  $0.27 \pm 0.03$ ,  $0.49 \pm 0.05$ ,  $0.36 \pm 0.04$ ,  $0.29 \pm 0.03$ ,  $F = 129.880$ ,  $P = 0.001$ )。模型组大鼠的 Cleaved caspase-3 蛋白表达量低于其余 5 组 ( $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ) , TLR4 蛋白表达量及 p-NF- $\kappa$ B p65/NF- $\kappa$ B p65 蛋白表达量比值均高于其余 5 组 ( $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ) ; 苦参碱低剂量组大鼠的 Cleaved caspase-3 蛋白表达量低于甲氨蝶呤组和苦参碱中、高剂量组 ( $P = 0.000$ ;  $P = 0.001$ ;  $P = 0.000$ ) , TLR4 蛋白表达量及 p-NF- $\kappa$ B p65/NF- $\kappa$ B p65 蛋白表达量比值均高于甲氨蝶呤组和苦参碱中、高剂量组 ( $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ) ; 苦参碱中剂量组大鼠的 Cleaved caspase-3 蛋白表达量低于甲氨蝶呤组和苦参碱高剂量组 ( $P = 0.000$ ;  $P = 0.000$ ) , TLR4 蛋白表达量及 p-NF- $\kappa$ B p65/NF- $\kappa$ B p65 蛋白表达量比值均高于甲氨蝶呤组和苦参碱高剂量组 ( $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ) ; 甲氨蝶呤组和苦参碱高剂量组大鼠 Cleaved caspase-3 蛋白、TLR4 蛋白表达量及 p-NF- $\kappa$ B p65/NF- $\kappa$ B p65 蛋白表达量比值的组间差异均无统计学意义 ( $P = 0.090$ ,  $P = 0.096$ ,  $P = 0.153$ )。结论: 苦参碱可有效减轻 RA 风湿热痹证大鼠的症状和体征, 且疗效存在剂量依赖性; 其作用机制可能是通过抑制 TLR4/NF- $\kappa$ B 信号通路, 减轻炎症反应、促进滑膜细胞凋亡。

**关键词** 关节炎, 类风湿; 风湿; 热痹; 苦参碱; Toll 样受体 4; NF- $\kappa$ B

## The therapeutic effects and mechanism of matrine on rheumatoid arthritis with wind - dampness - heat arthromyodynia via Toll - like receptor 4/nuclear factor - $\kappa$ B signaling pathway

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**ABSTRACT Objective:** To investigate the therapeutic effects and mechanism of matrine on rheumatoid arthritis (RA) with wind - dampness - heat arthromyodynia via Toll - like receptor 4 (TLR4)/nuclear factor  $\kappa$ B (NF- $\kappa$ B) signaling pathway. **Methods:** Sixty 8 - week - old specific pathogen - free (SPF) - grade male Sprague - Dawley (SD) rats were randomly assigned into normal group, RA model group, methotrexate group, matrine low - dose group, matrine medium - dose group and matrine high - dose group, 10 cases in each group. The rats in RA model group, methotrexate group, matrine low - dose group, matrine medium - dose group and matrine high - dose group were intervened by bovine type II collagen in artificial climate chamber for inducing RA with wind - dampness - heat arthromyodynia. After successful modeling, the rats in methotrexate group were intragastric administrated with methotrexate in dosage of 1.0 mg/kg, the ones in matrine low - , medium - and high - dose groups with matrine in dosages of 30 mg/kg, 60 mg/kg and 120 mg/kg respectively, and the ones in normal group and RA model group with the same dosage of distilled water, once a week for consecutive 4 times. The general condition of the rats was observed, the toe swelling degree and arthritis index were detected, and the synovial tissues of rat knee joints were stained with hematox-

lin-eosin (HE) for observing the pathological changes. Furthermore, the serum levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ) were detected by using enzyme linked immunosorbent assay (ELISA), the mRNA expression levels of Bax, Bcl-2, TLR4 and NF- $\kappa$ B p65 as well as the protein expression levels of Cleaved caspase-3, TLR4, NF- $\kappa$ B p65 and phosphorylated nuclear factor- $\kappa$ B p65 (p-NF- $\kappa$ B p65) in rat knee synovial tissues were detected by using real-time quantitative PCR (RT-qPCR) and Western-blot assays respectively. **Results:** ① Rats with smooth and shiny furs, normal eating and drinking as well as healthy toes were observed in normal group. After modeling, the rats with such symptoms as dry and lusterless furs, polydipsia, irritability, aggression and the abnormal toes, manifesting as swelling, red-heat, curled up and stiffness were observed in RA model group, methotrexate group, matrine low-dose group, matrine medium-dose group and matrine high-dose group, however, compared to RA model group, the symptoms of dry and lusterless furs as well as swollen toes were improved after drug intervention in rats of methotrexate group, matrine low-dose group, matrine medium-dose group and matrine high-dose group. ② There was statistical difference in toe swelling degree among the 6 groups ( $0.08 \pm 0.01, 0.51 \pm 0.07, 0.24 \pm 0.04, 0.44 \pm 0.06, 0.37 \pm 0.04, 0.28 \pm 0.06, F = 118.983, P = 0.000$ ). The degree of toe swelling in rats was higher in RA model group compared to the other five groups ( $P = 0.000; P = 0.000; P = 0.000; P = 0.000; P = 0.000$ ), and it was higher in the matrine low-dose group compared to methotrexate group, matrine medium-dose group and matrine high-dose group ( $P = 0.007; P = 0.000; P = 0.000$ ), and was higher in matrine medium-dose group compared to methotrexate group and matrine high-dose group ( $P = 0.001; P = 0.000$ ), however, the difference was not significant between methotrexate group and matrine high-dose group ( $P = 0.096$ ). ③ No abnormality was found in the toes of rats in the normal group with arthritis index evaluated as 0 point, while there was statistical difference in arthritis index among the other 5 groups ( $7.02 \pm 0.24, 4.36 \pm 0.12, 6.32 \pm 0.16, 5.58 \pm 0.20, 4.48 \pm 0.14$  points,  $F = 422.684, P = 0.000$ ). The arthritis index was higher in RA model group compared to methotrexate group, matrine low-dose group, matrine medium-dose group and matrine high-dose group ( $P = 0.000; P = 0.000; P = 0.000; P = 0.000$ ), and was higher in matrine low-dose group compared to methotrexate group, matrine medium-dose group and matrine high-dose group ( $P = 0.000; P = 0.000; P = 0.000$ ), and was higher in matrine medium-dose group compared to methotrexate group and matrine high-dose group ( $P = 0.000; P = 0.000$ ), however, the difference was not significant between methotrexate group and matrine high-dose group ( $P = 0.054$ ). ④ There was statistical difference in serum levels of TNF- $\alpha$  and IL-1 $\beta$  among the 6 groups (TNF- $\alpha$ :  $48.09 \pm 4.88, 351.49 \pm 32.39, 143.05 \pm 10.02, 281.51 \pm 26.50, 207.63 \pm 17.00, 154.40 \pm 14.23$  pg/mL,  $F = 311.253, P = 0.000$ ; IL-1 $\beta$ :  $30.71 \pm 4.60, 258.14 \pm 20.85, 105.27 \pm 10.38, 201.57 \pm 16.51, 158.97 \pm 16.18, 114.37 \pm 10.48$  pg/mL,  $F = 337.119, P = 0.000$ ). The serum levels of TNF- $\alpha$  and IL-1 $\beta$  were higher in RA model group compared to the other 5 groups ( $P = 0.000, P = 0.000; P = 0.000, P = 0.000; P = 0.000, P = 0.000; P = 0.000, P = 0.000$ ), and were higher in matrine low-dose group compared to methotrexate group, matrine medium-dose group and matrine high-dose group ( $P = 0.000, P = 0.000; P = 0.000, P = 0.000; P = 0.000, P = 0.000$ ), and were higher in matrine medium-dose group compared to methotrexate group and matrine high-dose group ( $P = 0.000, P = 0.000; P = 0.000, P = 0.000$ ), however, the difference was not significant between methotrexate group and matrine high-dose group ( $P = 0.054; P = 0.067$ ). ⑤ The HE staining results showed that the complete structure and orderly arranged cells without infiltration by inflammatory cells were observed in knee synovial tissues of rats from normal group; compared to normal group, the obvious proliferation and disorderly arranged cells infiltrated by a large number of inflammatory cells with smeared-out boundary were observed in knee synovial tissues of rats from RA model group; compared to RA model group, the reduced proliferation and the relatively well-arranged cells with improved inflammatory cell infiltration in varying degrees were observed in knee synovial tissues of rats from methotrexate group, matrine low-dose group, matrine medium-dose group and matrine high-dose group. ⑥ There was statistical difference in mRNA expression levels of Bax, Bcl-2, TLR4 and NF- $\kappa$ B p65 in rat knee synovial tissues among the 6 groups (Bax mRNA:  $0.80 \pm 0.07, 0.40 \pm 0.03, 0.72 \pm 0.08, 0.53 \pm 0.05, 0.63 \pm 0.05, 0.71 \pm 0.06, F = 69.870, P = 0.000$ ; Bcl-2 mRNA:  $0.19 \pm 0.02, 0.78 \pm 0.06, 0.33 \pm 0.03, 0.67 \pm 0.05, 0.54 \pm 0.06, 0.36 \pm 0.04, F = 258.197, P = 0.000$ ; TLR4 mRNA:  $0.13 \pm 0.01, 0.61 \pm 0.07, 0.25 \pm 0.02, 0.54 \pm 0.05, 0.45 \pm 0.04, 0.27 \pm 0.03, F = 206.811, P = 0.000$ ; NF- $\kappa$ B p65 mRNA:  $0.17 \pm 0.01, 0.56 \pm 0.04, 0.26 \pm 0.02, 0.46 \pm 0.04, 0.34 \pm 0.04, 0.28 \pm 0.03, F = 220.358, P = 0.000$ ). The mRNA expression level of Bax in rat knee synovial tissues was lower, while the mRNA expression levels of Bcl-2, TLR4 and NF- $\kappa$ B p65 in rat knee synovial tissues were higher in RA model group compared to the other 5 groups ( $P = 0.000; P = 0.000; P = 0.000; P = 0.000; P = 0.000; P = 0.000, P = 0.000, P = 0.000; P = 0.000, P = 0.019, P = 0.000; P = 0.000, P = 0.000, P = 0.000; P = 0.000, P = 0.000, P = 0.000; P = 0.000, P = 0.000, P = 0.000$ ). The mRNA expression level of Bax in rat knee synovial tissues was lower, while the mRNA expression levels of Bcl-2, TLR4 and NF- $\kappa$ B p65 in rat knee synovial tissues were higher in matrine low-dose group compared to methotrexate group, ma-

trine medium-dose group and matrine high-dose groups ( $P = 0.000; P = 0.000; P = 0.000; P = 0.000, P = 0.000, P = 0.000; P = 0.000, P = 0.000, P = 0.000; P = 0.000, P = 0.000, P = 0.000$ ). The mRNA expression level of Bax in rat knee synovial tissues was lower, while the mRNA expression levels of Bcl-2, TLR4 and NF- $\kappa$ B p65 in rat knee synovial tissues were higher in matrine medium-dose group compared to methotrexate group and matrine high-dose group ( $P = 0.000; P = 0.000; P = 0.000, P = 0.000, P = 0.000; P = 0.000, P = 0.000, P = 0.001$ ). However, there was no statistical difference in the mRNA expression levels of Bax, Bcl-2, TLR4 and NF- $\kappa$ B p65 in rat knee synovial tissues between methotrexate group and matrine high-dose group ( $P = 0.755, P = 0.074, P = 0.096, P = 0.096$ ).

⑦ There was statistical difference in protein expression levels of Cleaved caspase-3 and TLR4 as well as the ratio of protein expression level of p-NF- $\kappa$ B p65 to NF- $\kappa$ B p65 among the 6 group (Cleaved caspase-3 protein:  $0.74 \pm 0.06, 0.32 \pm 0.03, 0.62 \pm 0.05, 0.39 \pm 0.04, 0.47 \pm 0.05, 0.58 \pm 0.05, F = 127.351, P = 0.001$ ; TLR4 protein:  $0.17 \pm 0.02, 0.67 \pm 0.06, 0.25 \pm 0.03, 0.43 \pm 0.05, 0.35 \pm 0.03, 0.27 \pm 0.02, F = 216.610, P = 0.001$ ; the ratio of protein expression level of p-NF- $\kappa$ B p65 to NF- $\kappa$ B p65:  $0.24 \pm 0.02, 0.64 \pm 0.07, 0.27 \pm 0.03, 0.49 \pm 0.05, 0.36 \pm 0.04, 0.29 \pm 0.03, F = 129.880, P = 0.001$ ). The protein expression level of Cleaved caspase-3 was lower, while the protein expression level of TLR4 and the ratio of protein expression level of p-NF- $\kappa$ B p65 to NF- $\kappa$ B p65 were higher in RA model group compared to the other 5 groups ( $P = 0.000; P = 0.000; P = 0.000; P = 0.000; P = 0.000; P = 0.000, P = 0.000; P = 0.000, P = 0.000; P = 0.000, P = 0.000; P = 0.000, P = 0.000$ ). The protein expression level of Cleaved caspase-3 was lower, while the protein expression level of TLR4 and the ratio of protein expression level of p-NF- $\kappa$ B p65 to NF- $\kappa$ B p65 were higher in matrine low-dose group compared to methotrexate group, matrine medium-dose group and matrine high-dose group ( $P = 0.000; P = 0.001; P = 0.000; P = 0.000, P = 0.000; P = 0.000, P = 0.000; P = 0.000, P = 0.000$ ). The protein expression level of Cleaved caspase-3 was lower, while the protein expression level of TLR4 and the ratio of protein expression level of p-NF- $\kappa$ B p65 to NF- $\kappa$ B p65 were higher in matrine medium-dose group compared to methotrexate group and matrine high-dose group ( $P = 0.000; P = 0.000; P = 0.000, P = 0.000; P = 0.000, P = 0.000$ ). However, there was no statistical difference in the protein expression levels of Cleaved caspase-3 and TLR4 as well as the ratio of protein expression level of p-NF- $\kappa$ B p65 to NF- $\kappa$ B p65 between methotrexate group and matrine high-dose group ( $P = 0.090, P = 0.096, P = 0.153$ ).

**Conclusion:** The matrine can effectively improve the symptoms and physical signs in RA rat models with wind-dampness-heat arthromyodynia, and it exhibits dose-dependence in the efficacy. Its mechanisms may be that it can reduce inflammatory reaction and promote synovial cell apoptosis through inhibiting TLR4/Nf- $\kappa$ B signaling pathway.

**Keywords** arthritis, rheumatoid; wind dampness; arthralgia due to heat toxicity; matrine; Toll-like receptor 4; NF- $\kappa$ B

类风湿关节炎 (rheumatoid arthritis, RA) 是一种以滑膜炎为主要病理表现的慢性系统性疾病, 特征为手、足关节的对称性、侵袭性关节炎, 最终可导致关节畸形及功能丧失<sup>[1]</sup>。Toll 样受体 4 (Toll-like receptor 4, TLR4)/核因子  $\kappa$ B (nuclear factor- $\kappa$ B, NF- $\kappa$ B) 信号通路被认为与 RA 有关<sup>[2]</sup>, 调控该信号通路可作为 RA 的潜在治疗方向。RA 属中医学痹证范围, 风湿热痹证是其常见证候类型。苦参碱是从豆科植物苦参中提取的一种生物碱, 具有抗病毒、抗炎和调节免疫等作用<sup>[3-4]</sup>。本研究拟通过建立 RA 风湿热痹证大鼠模型, 研究苦参碱对 RA 风湿热痹证的治疗作用及作用机制, 为临床治疗 RA 风湿热痹证提供参考。

## 1 材料与仪器

**1.1 实验动物** 8 周龄 SPF 级雄性 SD 大鼠 60 只, 体重 180~200 g, 购自北京科宇动物养殖中心, 实

验动物合格证号为 SCXK(京)-2018-0010。适应性喂养 7 d 后开始实验。实验方案通过医院医学动物实验伦理委员会审查通过。

**1.2 主要试剂** 牛 II 型胶原 (北京博雷德生物技术有限公司), 弗氏完全佐剂 (美国 Sigma 公司), 甲氨蝶呤 (上海上药信谊药厂有限公司, 每片 2.5 mg), 苦参碱 (纯度  $\geq 98\%$ , 美国 Sigma 公司), 肿瘤坏死因子- $\alpha$  (tumor necrosis factor- $\alpha$ , TNF- $\alpha$ ) ELISA 试剂盒、白细胞介素-1 $\beta$  (interleukin-1 $\beta$ , IL-1 $\beta$ ) ELISA 试剂盒 (上海斯信生物科技有限公司), HE 染色液 (北京索莱宝生物科技有限公司), RNA 提取试剂盒 (美国 Invitrogen 公司), BCA 蛋白浓度测定试剂盒 (上海碧云天生物技术有限公司), 山羊抗兔二抗 IgG (上海碧云天生物技术有限公司), 兔抗大鼠 NF- $\kappa$ B p65 抗体、兔抗大鼠磷酸化核因子  $\kappa$ B p65 (phosphorylated nuclear factor- $\kappa$ B p65, p-NF- $\kappa$ B p65) 抗体、兔抗大

鼠 TLR4 抗体、兔抗大鼠 Cleaved Caspase-3 抗体、兔抗大鼠  $\beta$ -actin 抗体(美国 Cell Signaling Technology 公司)。

**1.3 主要仪器** YLS-7B 型足趾容积测量仪(上海欣软信息科技有限公司),生物组织自动脱水机、包埋机、石蜡切片机(德国 Leica 公司),722 型可见分光光度计(上海第三分析仪器厂),StepOnePlus 实时定量 PCR 仪(美国 Applied Biosystems 公司)。

## 2 方法

**2.1 分组及造模** 将 60 只大鼠随机分为 6 组,每组 10 只。通过牛 II 型胶原诱导<sup>[5]</sup>结合人工气候箱干预进行 RA 风湿热痹证造模。将 50 mg 牛 II 型胶原干粉溶于 25 mL 冰醋酸溶液( $0.1 \text{ mol} \cdot \text{L}^{-1}$ )中,再与 25 mL 弗氏完全佐剂混合,配制成胶原乳化液。模型组、甲氨蝶呤组及苦参碱低、中、高剂量组大鼠,按照牛 II 型胶原诱导法在背部脊柱两侧和尾根部分别注射胶原乳化液 0.1 mL;注射结束后当天开始,将大鼠置于人工气候箱中进行干预,设置温度  $36^\circ\text{C}$ 、湿度 95%、风速  $5 \text{ m} \cdot \text{s}^{-1}$ ,每天 1 次,每次 1 h,连续 7 d;第 8 天,在模型组、甲氨蝶呤组及苦参碱低、中、高剂量组大鼠左后肢足趾皮下注射 0.1 mL 胶原乳化液加强免疫,然后再放入人工气候箱继续干预(人工气候箱设置条件不变),每天 1 次,每次 1 h,连续 7 d。正常组大鼠仅于相同时间点,在相同部位注射等量生理盐水,常规环境饲养。

**2.2 药物干预** 自第 2 次人工气候箱干预开始后第 2 天起进行药物干预。甲氨蝶呤组大鼠按照  $1.0 \text{ mg} \cdot \text{kg}^{-1}$  以甲氨蝶呤(蒸馏水配制)灌胃,苦参碱低、中、高剂量组大鼠分别按照  $30 \text{ mg} \cdot \text{kg}^{-1}$ 、 $60 \text{ mg} \cdot \text{kg}^{-1}$ 、 $120 \text{ mg} \cdot \text{kg}^{-1}$  以苦参碱(蒸馏水配制)灌胃,正常组和模型组大鼠则以等体积蒸馏水灌胃。药物干预均每周 1 次,共干预 4 次。

## 2.3 实验指标检测

**2.3.1 大鼠一般情况观察** 实验期间,观察大鼠与 RA 风湿热痹证相关表现的变化情况,如毛发光泽、饮食、行为活动、足趾肿胀等情况。

**2.3.2 足趾肿胀度测定** 分别于造模前和药物干预结束后测定并计算大鼠的足趾肿胀度,具体方法如下:将大鼠左后足放入足趾容积测量仪,静止 3 s 后读数,测量 3 次取平均值,足趾肿胀度 = (药物干预结束后足趾体积 - 造模前足趾体积) / 造模前足趾体积。

**2.3.3 关节炎指数评定** 分别于造模前和药物干预结束后,采用 5 级评分法<sup>[5]</sup>评定大鼠的关节炎指数:0 分,正常;1 分,足趾关节轻度发红或肿胀;2 分,足趾关节以下部位肿胀;3 分,踝关节以下全部肿胀;4 分,整个足部肿胀或关节严重变形。以每只大鼠双后足评分之和作为最终评分。

**2.3.4 血清 TNF- $\alpha$ 、IL-1 $\beta$  含量测定** 药物干预结束后当天,以 2% 戊巴比妥钠麻醉大鼠,自腹主动脉取血 2 mL,在  $4^\circ\text{C}$  以  $3000 \text{ r} \cdot \text{min}^{-1}$  离心 10 min(离心半径 8 cm),收集上清液,于  $-80^\circ\text{C}$  条件下保存。以 ELISA 法检测血清 TNF- $\alpha$ 、IL-1 $\beta$  含量:包被抗体的聚苯乙烯酶标板经缓冲液清洗后,加入 5% 牛血清蛋白进行封闭;设置空白孔、标准孔和待测样品孔,标准孔加入稀释后的标准品 50  $\mu\text{L}$ 、待测样品孔加入稀释液 40  $\mu\text{L}$  后再加入待测样品 10  $\mu\text{L}$ ;除空白孔外,其余孔加辣根过氧化物酶标记的检测抗体 100  $\mu\text{L}$ ,  $37^\circ\text{C}$  孵育 1 h;洗涤,加显色剂 A 和 B 各 50  $\mu\text{L}$ ,  $37^\circ\text{C}$  孵育 20 min 后加终止液 50  $\mu\text{L}$ ,终止反应;在 450 nm 检测吸光度值,根据标准品浓度和吸光度值绘制标准曲线,计算各组大鼠血清 TNF- $\alpha$ 、IL-1 $\beta$  含量。

**2.3.5 膝关节滑膜组织病理学观察** 腹主动脉取血后,脱颈处死大鼠,仰卧位固定,乙醇消毒后沿膝关节正中切开皮肤,暴露长 3 cm、宽 3 cm 的区域,自髌骨上缘向下切开至股骨,再沿髌骨两侧向下分离至胫骨,剥离滑膜组织,部分在  $-80^\circ\text{C}$  保存、部分置于 4% 多聚甲醛溶液中固定。将在 4% 多聚甲醛溶液中固定 24 h 的滑膜组织取出,经脱水和透明处理后,以石蜡包埋切片,厚度 5  $\mu\text{m}$ ,HE 染色后置于显微镜下观察。

**2.3.6 膝关节滑膜组织 Bax mRNA、Bcl-2 mRNA、TLR4 mRNA、NF- $\kappa\text{B}$  p65 mRNA 表达量检测** 取部分于  $-80^\circ\text{C}$  保存的大鼠膝关节滑膜组织,以实时定量 PCR 法测定滑膜组织 Bax mRNA、Bcl-2 mRNA、TLR4 mRNA、NF- $\kappa\text{B}$  p65 mRNA 的表达量:以 RNA 提取试剂盒提取滑膜组织中目标基因的 RNA,通过逆转录酶逆转录得到 cDNA,以  $\beta$ -actin 作为内参。PCR 扩增条件为:预变性温度  $94^\circ\text{C}$ 、时间 5 min,变性温度  $94^\circ\text{C}$ 、时间 30 s,退火温度  $60^\circ\text{C}$ 、时间 30 s,延伸温度  $72^\circ\text{C}$ 、时间 1 min,共 40 个循环。以  $2^{-\Delta\Delta\text{Ct}}$  法计算目标基因 mRNA 表达量。引物序列见表 1。

表 1 实时定量 PCR 引物序列

引物名称	引物序列(5'—3')
Bax	上游引物 CAGGATGCGTCCACCAAGAA
	下游引物 CGTGTCCACGTCAGCAATCA
Bcl-2	上游引物 CATTGTCGGGCCCATGAAG
	下游引物 CACCCTGGCCCAATCTAGGA
Toll 样受体 4	上游引物 TTGCCTTCATTACAGGGACTT
	下游引物 AGATACCGGTGGAGGCTGACT
核因子 $\kappa$ B p65	上游引物 ACGATCTGTTCCTCCCTCATC
	下游引物 TGCTTCTCTCCCAGGAATA
$\beta$ -actin	上游引物 GCCATGTACGTAGCCATCCA
	下游引物 GAACCGCTCATGCCCATAG

**2.3.7 膝关节滑膜组织 Cleaved caspase-3 蛋白、TLR4 蛋白、NF- $\kappa$ B p65 蛋白、p-NF- $\kappa$ B p65 蛋白表达量检测** 取部分于  $-80^{\circ}\text{C}$  保存的大鼠膝关节滑膜组织,置于匀浆管中,加入 RIPA 裂解液裂解 20 min,  $4^{\circ}\text{C}$ 、 $12\,000\text{ r}\cdot\text{min}^{-1}$  离心 10 min (离心半径 8 cm),取上清液以 BCA 蛋白浓度测定试剂盒测定目标蛋白浓度。以 Western Blot 法测定 Cleaved caspase-3、TLR4、NF- $\kappa$ B p65、p-NF- $\kappa$ B p65 蛋白表达量:按  $20\text{ }\mu\text{g}$  蛋白量上样,10% 聚丙烯酰胺凝胶电泳 ( $80\text{ V}$  恒压电泳 20 min,  $100\text{ V}$  恒压电泳 90 min),  $300\text{ mA}$  恒流转膜 1 h, 5% 脱脂牛奶室温封闭 1 h, 滴加 NF- $\kappa$ B p65 (1:1000)、p-NF- $\kappa$ B p65 (1:1000)、TLR4 (1:1000)、Cleaved caspase-3 (1:1000)、 $\beta$ -actin (1:3000) 一抗,  $4^{\circ}\text{C}$  孵育过夜。洗膜后滴加 IgG 二抗 (1:3000), 室温孵育 1 h, 洗膜后电化学发光显色, Image J 软件测定灰度值,以  $\beta$ -actin 为内参计算目标蛋白相对表达量。

**2.4 数据统计** 采用 SPSS23.0 软件对数据进行统计分析。各组大鼠足趾肿胀度、关节炎指数、血清 TNF- $\alpha$  含量、血清 IL-1 $\beta$  含量及膝关节滑膜组织 Bax mRNA 表达量、Bcl-2 mRNA 表达量、TLR4 mRNA 表达量、NF- $\kappa$ B p65 mRNA 表达量、Cleaved

caspase-3 蛋白表达量、TLR4 蛋白表达量、NF- $\kappa$ B p65 与 p-NF- $\kappa$ B p65 蛋白表达量比值的组间总体比较均采用单因素方差分析,组间两两比较均采用 LSD- $t$  检验。检验水准  $\alpha=0.05$ 。

### 3 结果

**3.1 大鼠一般情况** 正常组大鼠毛发顺滑有光泽,饮食、饮水正常,足趾无肿胀;造模后模型组、甲氨蝶呤组及苦参碱低、中、高剂量组大鼠均出现毛发干燥无光泽,摄水量增加,易激惹、攻击性强,足趾肿胀、红肿、蜷缩、僵硬等表现;与模型组相比,药物干预后甲氨蝶呤组及苦参碱低、中、高剂量组大鼠毛发干燥无光泽、足趾肿胀等情况有所改善。

**3.2 足趾肿胀度** 6 组大鼠的足趾肿胀度比较,差异有统计学意义。模型组大鼠的足趾肿胀度高于其余 5 组 ( $P=0.000$ ;  $P=0.000$ ;  $P=0.000$ ;  $P=0.000$ ;  $P=0.000$ ),苦参碱低剂量组大鼠的足趾肿胀度高于甲氨蝶呤组和苦参碱中、高剂量组 ( $P=0.007$ ;  $P=0.000$ ;  $P=0.000$ ),苦参碱中剂量组大鼠的足趾肿胀度高于甲氨蝶呤组和苦参碱高剂量组 ( $P=0.001$ ;  $P=0.000$ ),甲氨蝶呤组和苦参碱高剂量组大鼠足趾肿胀度的差异无统计学意义 ( $P=0.096$ )。见表 2。

**3.3 关节炎指数** 正常组大鼠足部未见异常,关节炎指数为 0 分;其余 5 组大鼠关节炎指数比较,差异有统计学意义。模型组大鼠的关节炎指数高于甲氨蝶呤组和苦参碱低、中、高剂量组 ( $P=0.000$ ;  $P=0.000$ ;  $P=0.000$ ),苦参碱低剂量组大鼠的关节炎指数高于甲氨蝶呤组和苦参碱中、高剂量组 ( $P=0.000$ ;  $P=0.000$ ;  $P=0.000$ ),苦参碱中剂量组大鼠的关节炎指数高于甲氨蝶呤组和苦参碱高剂量组 ( $P=0.000$ ;  $P=0.000$ ),甲氨蝶呤组和苦参碱高剂量组大鼠关节炎指数的差异无统计学意义 ( $P=0.054$ )。见表 2。

表 2 6 组大鼠的足趾肿胀度和关节炎指数

组别	样本量/只	足趾肿胀度 ( $\bar{x} \pm s$ )	关节炎指数 ( $\bar{x} \pm s$ , 分)
正常组	10	$0.08 \pm 0.01$	0
模型组	10	$0.51 \pm 0.07$	$7.02 \pm 0.24$
甲氨蝶呤组	10	$0.24 \pm 0.04$	$4.36 \pm 0.12$
苦参碱低剂量组	10	$0.44 \pm 0.06$	$6.32 \pm 0.16$
苦参碱中剂量组	10	$0.37 \pm 0.04$	$5.58 \pm 0.20$
苦参碱高剂量组	10	$0.28 \pm 0.06$	$4.48 \pm 0.14$
$F$ 值		118.983	422.684 <sup>1)</sup>
$P$ 值		0.000	0.000 <sup>1)</sup>

1) 正常组关节炎指数为 0 分,仅对其余 5 组的数据进行单因素方差分析。



**3.4 血清 TNF- $\alpha$ 、IL-1 $\beta$  含量** 6 组大鼠的血清 TNF- $\alpha$ 、IL-1 $\beta$  含量比较,组间差异均有统计学意义。模型组大鼠的血清 TNF- $\alpha$ 、IL-1 $\beta$  含量均高于其余 5 组 ( $P = 0.000, P = 0.000; P = 0.000, P = 0.000; P = 0.000, P = 0.000; P = 0.000, P = 0.000$ ),苦参碱低剂量组大鼠的血清 TNF- $\alpha$ 、IL-1 $\beta$  含量均高于甲氨蝶呤组和苦参碱中、高剂量组 ( $P = 0.000, P = 0.000; P = 0.000, P = 0.000; P = 0.000, P = 0.000$ ),苦参碱中剂量组大鼠的血清 TNF- $\alpha$ 、IL-1 $\beta$  含量均高于甲氨蝶呤组和苦参碱高剂量组 ( $P = 0.000, P = 0.000; P = 0.000, P =$

$0.000$ ),甲氨蝶呤组和苦参碱高剂量组大鼠血清 TNF- $\alpha$ 、IL-1 $\beta$  含量的差异均无统计学意义 ( $P = 0.054; P = 0.067$ )。见表 3。

**3.5 膝关节滑膜组织病理学观察结果** HE 染色结果显示,正常组大鼠膝关节滑膜组织结构完整,细胞排列整齐,无炎症细胞浸润;与正常组相比,模型组大鼠滑膜组织增生明显,有大量炎症细胞浸润,滑膜细胞排列紊乱,边界模糊不清;与模型组相比,甲氨蝶呤组和苦参碱低、中、高剂量组滑膜组织增生程度减轻,滑膜细胞排列较整齐,炎症细胞浸润情况均得到不同程度改善(图 1)。

表 3 6 组大鼠的血清肿瘤坏死因子- $\alpha$  和白细胞介素-1 $\beta$  含量

组别	样本量/只	肿瘤坏死因子- $\alpha$ /( $\bar{x} \pm s, \text{pg} \cdot \text{mL}^{-1}$ )	白细胞介素-1 $\beta$ /( $\bar{x} \pm s, \text{pg} \cdot \text{mL}^{-1}$ )
正常组	10	48.09 $\pm$ 4.88	30.71 $\pm$ 4.60
模型组	10	351.49 $\pm$ 32.39	258.14 $\pm$ 20.85
甲氨蝶呤组	10	143.05 $\pm$ 10.02	105.27 $\pm$ 10.38
苦参碱低剂量组	10	281.51 $\pm$ 26.50	201.57 $\pm$ 16.51
苦参碱中剂量组	10	207.63 $\pm$ 17.00	158.97 $\pm$ 16.18
苦参碱高剂量组	10	154.40 $\pm$ 14.23	114.37 $\pm$ 10.48
<i>F</i> 值		311.253	337.119
<i>P</i> 值		0.000	0.000

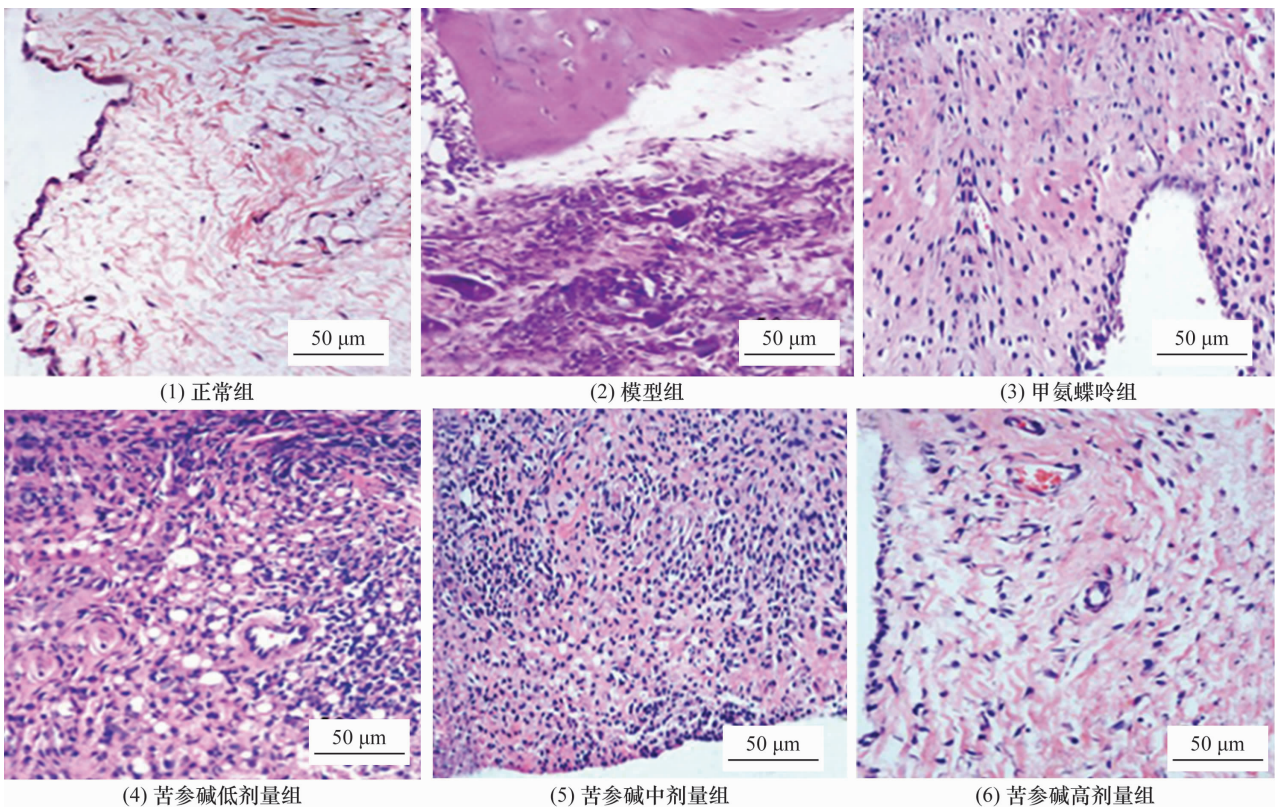


图 1 6 组大鼠膝关节滑膜组织 HE 染色图( $\times 200$ )

**3.6 膝关节滑膜组织 Bax mRNA、Bcl - 2 mRNA、TLR4 mRNA、NF -  $\kappa$ B p65 mRNA 表达量** 6 组大鼠膝关节滑膜组织 Bax mRNA、Bcl - 2 mRNA、TLR4 mRNA、NF -  $\kappa$ B p65 mRNA 表达量比较,组间差异均有统计学意义。模型组大鼠的膝关节滑膜组织 Bax mRNA 表达量低于其余 5 组 ( $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ), Bcl - 2 mRNA、TLR4 mRNA、NF -  $\kappa$ B p65 mRNA 表达量均高于其余 5 组 ( $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.019$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ );苦参碱低剂量组大鼠的膝关节滑膜组织 Bax mRNA 表达量低于甲氨蝶呤组和苦参碱中、高剂量组 ( $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ), Bcl - 2 mRNA、TLR4 mRNA、NF -  $\kappa$ B p65 mRNA 表达量均高于甲氨蝶呤组及苦参碱中、高剂量组 ( $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ );苦参碱中剂量组大鼠的膝关节滑膜组织 Bax mRNA 表达量低于甲氨蝶呤组和苦参碱高剂量组 ( $P = 0.000$ ;  $P = 0.000$ ), Bcl - 2 mRNA、TLR4 mRNA、NF -  $\kappa$ B p65 mRNA 表达量均高于甲氨蝶呤组和苦参碱高剂量组 ( $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ,  $P = 0.000$ );甲氨蝶呤组和苦参碱高剂量组大鼠膝关节滑膜组织 Bax mRNA、Bcl - 2 mRNA、TLR4 mRNA、NF -  $\kappa$ B p65 mRNA 表达量的组间差异均无统计学意义 ( $P = 0.755$ ,  $P = 0.074$ ,  $P = 0.096$ ,  $P = 0.096$ )。见表 4。

### 3.7 膝关节滑膜组织 Cleaved caspase-3 蛋白、TLR4 蛋白、NF- $\kappa$ B p65 蛋白、p-NF- $\kappa$ B p65 蛋白表达量

蛋白表达量及 p - NF -  $\kappa$ B p65/NF -  $\kappa$ B p65 蛋白表达量比值比较,组间差异均有统计学意义。模型组大鼠的 Cleaved caspase - 3 蛋白表达量低于其余 5 组 ( $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ;  $P = 0.000$ ), TLR4 蛋白表达量及 p - NF -  $\kappa$ B p65/NF -  $\kappa$ B p65 蛋白表达量比值均高于其余 5 组 ( $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ); 苦参碱低剂量组大鼠的 Cleaved caspase - 3 蛋白表达量低于甲氨蝶呤组和苦参碱中、高剂量组 ( $P = 0.000$ ;  $P = 0.001$ ;  $P = 0.000$ ), TLR4 蛋白表达量及 p - NF -  $\kappa$ B p65/NF -  $\kappa$ B p65 蛋白表达量比值均高于甲氨蝶呤组和苦参碱中、高剂量组 ( $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ); 苦参碱中剂量组大鼠的 Cleaved caspase - 3 蛋白表达量低于甲氨蝶呤组和苦参碱高剂量组 ( $P = 0.000$ ;  $P = 0.000$ ), TLR4 蛋白表达量及 p - NF -  $\kappa$ B p65/NF -  $\kappa$ B p65 蛋白表达量比值均高于甲氨蝶呤组和苦参碱高剂量组 ( $P = 0.000$ ,  $P = 0.000$ ;  $P = 0.000$ ,  $P = 0.000$ ); 甲氨蝶呤组和苦参碱高剂量组大鼠 Cleaved caspase - 3 蛋白、TLR4 蛋白表达量及 p - NF -  $\kappa$ B p65/NF -  $\kappa$ B p65 蛋白表达量比值的组间差异均无统计学意义 ( $P = 0.090$ ,  $P = 0.096$ ,  $P = 0.153$ )。见表 5、图 2。

## 4 讨 论

风湿热痹证是痹证的常见证候类型,其病邪性质为风、湿、热三邪<sup>[6]</sup>。活动期 RA 的证候以风湿热痹证为主,患者多见关节红肿、灼热、疼痛、喜冷恶热<sup>[7]</sup>。因此,治疗应以清热利湿,疏风止痛为主<sup>[8]</sup>。苦参碱具有清热解燥、去湿排毒以及抗炎等功效。研究发现,苦参碱能够减轻RA患者的压痛感,降低炎症因

表 4 6 组大鼠膝关节滑膜组织 Bax mRNA、Bcl-2 mRNA、TLR4 mRNA、NF- $\kappa$ B p65 mRNA 表达量

组别	样本量/ 只	Bax mRNA ( $\bar{x} \pm s$ )	Bcl-2 mRNA ( $\bar{x} \pm s$ )	TLR4 mRNA <sup>1)</sup> ( $\bar{x} \pm s$ )	NF- $\kappa$ B p65 mRNA <sup>2)</sup> ( $\bar{x} \pm s$ )
正常组	10	0.80 $\pm$ 0.07	0.19 $\pm$ 0.02	0.13 $\pm$ 0.01	0.17 $\pm$ 0.01
模型组	10	0.40 $\pm$ 0.03	0.78 $\pm$ 0.06	0.61 $\pm$ 0.07	0.56 $\pm$ 0.04
甲氨蝶呤组	10	0.72 $\pm$ 0.08	0.33 $\pm$ 0.03	0.25 $\pm$ 0.02	0.26 $\pm$ 0.02
苦参碱低剂量组	10	0.53 $\pm$ 0.05	0.67 $\pm$ 0.05	0.54 $\pm$ 0.05	0.46 $\pm$ 0.04
苦参碱中剂量组	10	0.63 $\pm$ 0.05	0.54 $\pm$ 0.06	0.45 $\pm$ 0.04	0.34 $\pm$ 0.04
苦参碱高剂量组	10	0.71 $\pm$ 0.06	0.36 $\pm$ 0.04	0.27 $\pm$ 0.03	0.28 $\pm$ 0.03
<i>F</i> 值		69.870	258.197	206.811	220.358
<i>P</i> 值		0.000	0.000	0.000	0.000

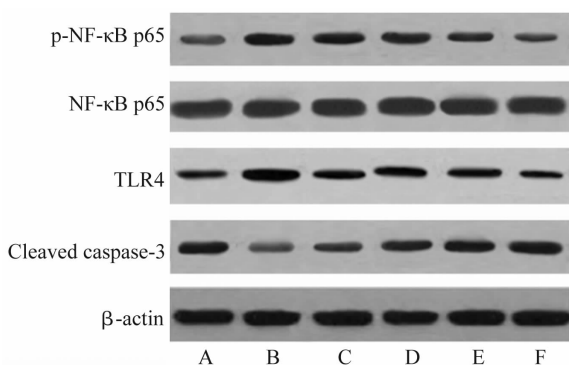
1)TLR4 为 Toll 样受体 4;2)NF- $\kappa$ B p65 为核因子  $\kappa$ B p65。



表 5 各组大鼠膝关节滑膜组织 Cleaved caspase-3 蛋白、TLR4 蛋白表达量  
及 NF-κB p65 蛋白/p-NF-κB p65 蛋白表达量比值

组别	样本量/ 只	Cleaved caspase-3 蛋白( $\bar{x} \pm s$ )	TLR4 <sup>1)</sup> 蛋白 ( $\bar{x} \pm s$ )	p-NF-κB p65 蛋白/NF-κB p65 蛋白 <sup>2)</sup> ( $\bar{x} \pm s$ )
正常组	10	0.74 ± 0.06	0.17 ± 0.02	0.24 ± 0.02
模型组	10	0.32 ± 0.03	0.67 ± 0.06	0.64 ± 0.07
甲氨蝶呤组	10	0.62 ± 0.05	0.25 ± 0.03	0.27 ± 0.03
苦参碱低剂量组	10	0.39 ± 0.04	0.43 ± 0.05	0.49 ± 0.05
苦参碱中剂量组	10	0.47 ± 0.05	0.35 ± 0.03	0.36 ± 0.04
苦参碱高剂量组	10	0.58 ± 0.05	0.27 ± 0.02	0.29 ± 0.03
F 值		127.351	216.610	129.880
P 值		0.001	0.001	0.001

1) Toll 样受体 4; 2) 中 p-NF-κB p65 为磷酸化核因子 κB p65, NF-κB p65 为核因子 κB p65。



p-NF-κB p65 为磷酸化核因子 κB p65; NF-κB p65 为核因子 κB p65; TLR4 为 Toll 样受体 4; A 为正常组; B 为模型组; C 为苦参碱低剂量组; D 为苦参碱中剂量组; E 为苦参碱高剂量组; F 为甲氨蝶呤组。

图 2 6 组大鼠膝关节滑膜组织中相关蛋白表达电泳图  
子水平<sup>[9]</sup>。苦参碱具有多种药理学活性,不仅可抑制  
宫颈鳞癌细胞增殖、迁移和侵袭,而且可减轻前列腺  
炎大鼠炎症反应<sup>[10-11]</sup>。Niu 等<sup>[12]</sup>研究发现,苦参碱  
可通过抑制 NF-κB 信号通路,调控 RA 中 Th1/Th2  
细胞因子应答。还有研究发现,苦参碱可通过抑制磷  
脂酰肌醇 3 激酶/蛋白激酶 B 信号通路,抑制胶原诱  
导的关节炎大鼠滑膜血管生成<sup>[13]</sup>。

本研究采用牛 II 型胶原诱导联合人工气候箱干  
预,建立 RA 风湿热痹证大鼠模型。研究结果显示,  
模型组大鼠足趾肿胀度和关节炎指数较正常组明显  
升高,且滑膜组织增生明显,有大量炎症细胞浸润,滑  
膜细胞排列紊乱,滑膜边界弥散不清,提示模型制备  
成功。经苦参碱干预后,RA 大鼠足趾肿胀度和关节  
炎指数明显降低,滑膜增生和炎症浸润情况得到改  
善,且改善效果存在剂量依赖性,但高剂量苦参碱的  
治疗效果与甲氨蝶呤无差别,提示苦参碱对 RA 风湿  
热痹证具有较好的治疗作用。本研究还发现,模型组

大鼠血清 TNF-α 和 IL-1β 含量较正常组升高,干  
预后 RA 大鼠血清 TNF-α 和 IL-1β 含量降低,提示  
苦参碱可通过减轻 RA 大鼠的炎症反应改善其关节  
肿胀。

TLR4/NF-κB 是免疫炎症损伤的重要信号通  
路,参与 RA 的发生发展<sup>[14-15]</sup>。NF-κB 的激活可诱  
导 TNF-α、IL-1β 等炎症因子的释放<sup>[16-17]</sup>。实时  
定量 PCR 和 Western Blot 结果显示,模型组大鼠膝关  
节滑膜组织 TLR4 mRNA、NF-κB p65 mRNA、TLR4  
蛋白表达量均较正常组升高;而与模型组相比,苦参  
碱各剂量组的上述指标均明显降低。这表明 TLR4/  
NF-κB 信号通路参与了 RA 的发生发展,苦参碱可  
能通过调控 TLR4/NF-κB 信号通路缓解 RA 的症状  
和体征。这与曲道炜等<sup>[18]</sup>的研究结论一致。p-  
NF-κB p65/NF-κB p65 蛋白表达量比值反映了  
NF-κB p65 蛋白的活化情况,模型组大鼠滑膜组织  
中该比值较正常组升高,说明 NF-κB p65 蛋白异常  
活化,TLR4/NF-κB 信号通路被激活;苦参碱各剂量  
组该比值较模型组降低,说明苦参碱可抑制 TLR4/  
NF-κB 信号通路。TLR4/NF-κB 信号通路的活化不  
仅会加剧炎症反应,还可促进滑膜细胞大量增殖,造  
成滑膜成纤维细胞凋亡不足<sup>[10,19]</sup>。Cleaved caspase-3  
是 Caspase-3 的活化形式,可诱导多种蛋白或酶类失  
活,改变细胞结构,加速细胞凋亡<sup>[11]</sup>。朱艳媚等<sup>[20]</sup>认  
为,可通过促进滑膜细胞凋亡减轻 RA 大鼠症状。本  
研究中,经苦参碱治疗后,RA 大鼠滑膜组织 Bax mR-  
NA 和 Cleaved caspase-3 蛋白表达水平均明显升高,  
Bcl-2 mRNA 表达水平明显降低,提示苦参碱可促进  
滑膜细胞凋亡。

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E1201 – E1205.

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(收稿日期: 2022-04-07 本文编辑: 吕宁)

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本研究的结果提示, 苦参碱可有效减轻 RA 风湿热痹证大鼠的症状和体征, 且疗效存在剂量依赖性; 其作用机制可能是通过抑制 TLR4/NF- $\kappa$ B 信号通路, 减轻炎症反应, 促进滑膜细胞凋亡。

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(收稿日期: 2022-03-03 本文编辑: 李晓乐)