·论著·

TNF-α、iNOS 在中药补肾固筋方治疗羊膝骨性关节炎中的表达及修复意义

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摘要:**目的** 研究补肾固筋方对骨关节炎(knee osteoarthritis,KOA)TNF-α、iNOS 的作用调节机制。**方法** 0.3 岁雄性山羊 72 只,随机分为正常组、模型组、西药组、中药组,每组 18 只,改良 Hulth 造模,取血清及股骨内髁关节软骨滑膜及软骨下骨,ELISA 法检测 TNF-α、iNOS。**结果** 西药组关节间隙狭窄,介于模型组和中药组之间,关节软骨面外观粗糙骨赘增生;中药组关节间隙稍窄,关节面稍粗糙伴轻微骨赘;镜下模型组软骨细胞呈成簇现象增生,中药组软骨细胞大小均匀且排列整齐;TNF-α、iNOS 含量模型组中比正常组增高($P<0.01$)。两个给药组比模型组明显降低($P<0.01$),两给药组之间无差异统计学意义($P>0.05$)。**结论** 补肾固筋方有效降低骨关节炎 TNF-α、iNOS 含量,促进软骨修复,提供实验依据。

关键词:补肾固筋方;羊膝骨性关节炎;软骨细胞;关节滑液;肿瘤坏死因子-α;诱导型一氧化氮合酶

Expression and significance of tumor necrosis factor-alpha and inducible nitric oxide synthase in knee osteoarthritis treated with TCM tonifying kidney and consolidating tendon prescription in goats

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Abstract: **Objective** To explore the regulation effect of tonifying kidney and consolidating tendon prescription on tumor necrosis factor-alpha (TNF-α) and inducible nitric oxide synthase (iNOS) in knee osteoarthritis (KOA) goats. **Methods** A total of 72 3-month-old healthy male goats were randomly divided into normal group, model group, Western medicine group, and TCM groups, with 18 goats in each group. The model was established using modified Hulth method. Serum and the cartilage, synovial membrane, and the subchondral bone of the femur and the medial malleolus were collected. TNF-α and iNOS were detected using ELISA method. **Results** The joint space narrowed in Western medicine group, and it was between the model group and the TCM group. The articular surface became roughness and the osteophyte developed. The joint space slightly narrowed in the TCM group, and the articular surface was slightly rough with mild osteophytes. The chondrocytes proliferated in a cluster manner with even size and good order under the microscope. The expression of TNF-α and iNOS was significantly higher in the model group than that in the normal group ($P<0.01$). It was significantly lower in both medicine groups than in the model group ($P<0.01$). The difference between the two medicine groups was not significant ($P>0.05$). **Conclusion** The tonifying kidney and consolidating tendon prescription effectively decreases the content of TNF-α and iNOS in KOA and promotes the repair of the cartilage. This provides experimental evidence for the clinical treatment.

Key words: Tonifying kidney and consolidating tendon prescription; Goat knee osteoarthritis; Chondrocytes; Joint synovial fluid; Tumor necrosis factor-α; Inducible nitric oxide synthase

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膝骨性关节炎(knee osteoarthritis, KOA)以关节软骨退变和骨质增生为病理特征的老年性慢性疾病[1]。《素问·痹论》:风寒湿三气杂至,合而为痹。中药熟地、枸杞、杜仲、牛膝等补益肝肾、强筋壮骨治疗 KOA 疗效确切[2,3]。补肾固筋方通过检测羊血清及关节液中 TNF-α、iNOS 的含量来明确其作用机制。

1 材料和方法

1.1 实验动物

0.3 岁雄性山羊 72 只,清洁级,体重 >15 kg[动物质量合格证号:SYXK(沪)2013-0036, scxk(冀)2013-11-004]。

1.2 药物

补肾固筋方组成:熟地 15 g、补骨脂 15 g、杜仲 12 g、巴戟天 10 g、川牛膝 12 g、丹参 12 g、桂枝 10 g、鸡血藤 12 g。根据进食、精神、喜暖恶寒,活动等中医辨证,风寒湿痹加防风、黄芪;风湿热痹加秦艽、羌活;瘀血闭阻加桃仁、红花;肝肾阴虚者加枸杞子、山茱萸等。常规煎煮取汁 300 mL。药液浓缩加入蒸馏水后配制成 6 ~ 8 g/10 mL;美洛昔康胶囊,7.5 mg/粒,四川宝光药业股份有限公司,国药准字:H20011219 批号:120502770065,加蒸馏水配成 0.5 mg/mL 溶液。

1.3 主要仪器及试剂

山羊 TNF-α、iNOS ELISA 试剂盒(96T, US. Abcam)、酶联免疫检测仪(Bio-565)、DNM-9602A 酶标分析仪(DNM-9602A)、干燥箱(TG100, Germany RETSCH Company);恒温水箱(DDIL-5 型,上海安亭科学仪器厂);石蜡切片机(U-641 型,美国贝克曼公司);光学显微镜照相系统(Leica, Germany)。

1.4 实验设计

除正常组外均采用改良 Hulth 造模[4]。完整切除内侧半月板、ACL(anterior cruciate ligament), MCL(medial collateral ligament),造成负重力线内移及膝关节不稳,术侧肢体不固定肌注青霉素 160 万 IU/(kg·d),连续 7 d,每隔 1d 观察创口愈合情况,预防感染。术后第 4 天开始强迫羊奔跑(0.5 h/d)。造模 2 w 软骨改变,6 w 后进入平台期,10 w 出现典型的晚期 OA 病理改变。当胫股关节面由正常外翻 10°变为内翻,X 线片下肢力线明显改变且跛行明显,前抽屉实验、内侧应力实验阳性即表示造模成功,本实验造模成功率为 90%。

造模 3 w 后开始干预,按羊每日药量(20 kg) = 成人(65 kg)每日药量 ×0.08 ×2.0 ×2/3,美洛昔康 6 mg/(kg·d),补肾固筋方 53 g/(kg·d),正常组、模型组灌胃 15 mL 生理盐水,2 次/天,连续干预 8 w。

1.5 观察指标

1.5.1 步态观察:David Coderre 法[5]步态分级[7]评价。0 级:正常行走,行走时间 >5 min; Ⅰ级:轻微跛行,行走时间 3 ~5 min; Ⅱ级:中度跛行,行走时间 <3 min。

1.5.2 X 线检查:按改良 David T[6]法分级,0 度:未见异常; Ⅰ度:关节间隙正常但关节面模糊; Ⅱ度:关节间隙狭窄; Ⅲ度:内外侧关节间隙严重狭窄不等宽。

1.5.3 形态学观察及 TNF-α、iNOS 检测:取血液离心后血清;膝关节前方部分滑膜组织和股骨内髁关节软骨及软骨下骨,剪成约 0.6 cm ×0.6 cm ×0.6 cm 组织块,切片。染色. 观察关节软骨的层次、软骨细胞变化及含量。

1.6 统计学处理

SPSS17.0 统计软件,数据 $\bar{x} \pm s$ 表示,组间比较 ANOSVA 检验,各样本均数间两两比较 q 检验,$P < 0.05$ 差异有统计学意义。

2 结果

2.1 肉眼观察

正常组软骨蓝白色,色泽透明,触之较硬,关节液量少;模型组滑膜肿胀,关节边缘与滑膜有少许纤维性粘连凹凸不平;两个给药组关节软骨呈白色,欠光滑,滑膜轻度增生,关节液较正常组稍多,滑膜炎性表现较模型组轻。

2.2 DR 观察

正常组羊膝关节内外侧间隙均匀一致,关节面平整,边缘整齐规则无骨赘,关节内无游离体;模型组膝关节内侧间隙变窄外侧间隙增大,骨囊变、骨赘形成;中药组膝关节内侧间隙狭窄不明显,未见明显骨赘形成;西药组膝关节内侧间隙介于模型组和中药组之间,有轻微骨赘形成。

2.3 形态学及 TNF-α、iNOS 含量(表 1)

正常组蓝白色透明,表面光滑较硬,关节液量少;模型组滑膜肿胀表面不光滑有少许纤维性粘连镜下软骨细胞排列不完整,深层簇聚;西药和中药呈白色欠光滑,滑膜轻度增生,关节液较正常组稍多,模型组软骨细胞排列欠完整簇状增生;中药组软骨细胞排列相对整齐。模型组 TNF-α、iNOS 含量较正常组升高 ($P < 0.01$)。两治疗组与模型组比较含

量降低（$P<0.01$），中药组含量较西药组分别高 （$P>0.05$）。2.3%、13.92%。两给药组差异无统计学意义

表 1 各组血清及关节液 TNF-α、iNOS 含量比较（TNF-α：pg/ml，iNOS：μmol/g，$\bar{x}\pm s$）

Table 1 Comparison of TNF-α and iNOS content in serum and synovial fluid among each group (TNF-α, pg/ml; iNOS, μmol/g, $\bar{x}\pm s$)

组别 (group)	例数 (n)	血清 (Serum)		关节液 (synovial fluid)	
		TNF-α	iNOS	TNF-α	iNOS
正常组 normal	18	523.1±41.0*	211.9±20.8*	51.3 ±0.9*	41.7±0.4*
模型组 model	18	894.2±31.5*	509.4±26.4*	98.6±0.4*	100.4±0.7*
中药组 Chinese medicine	18	678.4±38.4*	411.6±24.7*	65.2±0.7*	89.7±0.4*
西药组 western medicine	18	650.1±35.3*	352.7±16.8*	67.3 ±0.6*	76.4±0.5*

注：与模型组比较，* $P<0.01$；Note: compared with the model group, * $P<0.01$。

3 讨论

Wood[7] 1983 年首先报道了类风湿性关节炎（rheumatoid arthritis, RA）和骨性关节炎（osteoarthritis, OA）中都检测到 TNF-α、iNOS 的存在。TNF-α 诱发慢性滑膜炎，软骨基质降解产物的刺激也可引起滑膜的继发炎症[8]，促进软骨细胞分泌前列腺素（Prostaglandins 2, PGE_2）[9]，刺激骨吸收及成骨细胞增生、钙化，软骨下骨增厚骨赘生成[10]。OA 软骨基质细胞中 iNOS 及受体均呈阳性反应，强度与范围和 OA 严重程度正相关[11]；iNOS 刺激滑膜细胞 PGE_2 分泌，增加骨软骨破坏，TNF-α、iNOS 促进滑膜成纤维细胞样细胞增殖，加快膝关节软骨基质的破坏[12]。

治则补益肝肾、强筋健骨，熟地、补骨脂为君药，熟地性温味甘、滋阴养血，补骨脂补肾壮阳益精填髓之要药，熟地、补骨脂和枸杞子配伍改善微循环与血液流变学，降低骨内压，缓解骨和骨髓血流动力学引起的代谢异常；臣药巴戟天、川牛膝和鸡血藤改善软骨细胞功能，刺激软骨破坏区产生大量幼稚软骨[13]，减少或阻断因软骨片丢失刺激滑膜分泌，木瓜、丹参活血化瘀药促进关节内外的血液循环，改善静脉瘀滞状态降低骨内压[14]。研究中 12w 后羊膝关节积液明显减少，X 线示骨端变性、软骨下骨质萎缩，间隙增宽，对照组相反。补肾固筋方通过降低 TNF-α、iNOS 的分泌抑制滑膜炎性改变，减少炎性物质对软骨和滑膜的侵害，同时滑膜渗透作用于软骨细胞增强软骨细胞的代偿能力，对软骨破坏起到一定延缓作用，延缓软骨退变促进软骨修复。

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(上接第 673 页)

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