

·基础研究·

基于细胞外信号调节激酶通路探讨筋滞骨错手法对兔膝关节炎软骨退变的影响及作用机制

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摘要 目的 基于细胞外信号调节激酶(ERK)通路探讨筋滞骨错手法对兔膝关节炎(KOA)软骨退变的影响, 并分析其作用机制。**方法** 选择健康雄性新西兰大白兔 30 只, 采用随机数字表法分为空白组和造模组, 分别 10、20 只。空白组不做任何处理, 造模组参照 Videman 造模方法, 制备 KOA 早期模型。由于造模失败和干预期间死亡等原因, 空白组和造模组最后分别纳入 8 只、16 只。造模组按随机数字表法分为模型组和手法组, 每组 8 只。空白组、模型组自由活动和饮食, 不进行任何干预; 手法组在造模成功后开始进行筋滞骨错手法治疗, 包括理筋滞手法(顺足三阳经、三阴经; 点按伏兔、梁丘、内外膝眼、血海、阳陵泉、阿是穴等穴位, 60~80 次/min, 每穴点按 2 min, 12 min/次, 1 次/d, 共干预 21 d)和纠骨错手法(1 次/周, 3 次/疗程, 共干预 21 d)。采用 Lequesne MG 分级量表评价行为学与步态; 采用苏木精-伊红(HE)染色法观察兔膝关节病理学改变; 采用番红 O-固绿染色法观察关节软骨及软骨下骨的组织结构变化; 采用 Western blot 检测 ERK1/2、磷酸化激活的 ERK1/2(pERK1/2)占比、基质金属蛋白酶 13(MMP-13)和肿瘤坏死因子- α (TNF- α)的蛋白表达水平; 采用定量逆转录聚合酶链式反应(qRT-PCR)法检测 ERK1/2、MMP-13、白细胞介素-1 β (IL-1 β)、TNF- α mRNA 转录水平; 采用 Micro CT 检测兔膝关节骨小梁连接密度(Conn.D)、骨小梁数量(Tb.N)。**结果** ① Lequesne MG 评分: 与空白组比较, 模型组和手法组干预后 Lequesne MG 评分均明显升高($P<0.05$)。与模型组比较, 手法组干预后 Lequesne MG 评分明显降低($P<0.05$)。② 软骨病理学改变: 模型组内侧平台表面软骨不平整, 局部可见软骨面的丢失, 浅层软骨细胞减少, 呈肥大分化, 深层软骨细胞聚集成团; 可见明显软骨裂隙及缺损, 软骨基质着色不均匀。手法组表层软骨较模型组平整, 细胞排列分布较均匀; 软骨基质染色较均匀。③ TNF- α 、MMP-13、ERK1/2 蛋白表达水平和 pERK1/2 占比: 与空白组比较, 模型组干预后 TNF- α 、MMP-13、ERK1/2 蛋白表达水平和 pERK1/2 占比均明显升高($P<0.05$)。与模型组比较, 手法组干预后 TNF- α 、MMP-13、ERK1/2 蛋白表达水平和 pERK1/2 占比均明显降低($P<0.05$)。④ MMP-13、IL-1 β 、TNF- α 和 ERK1/2 mRNA 转录水平: 与空白组比较, 模型组干预后 TNF- α 、MMP-13 和 IL-1 β mRNA 转录水平均明显升高($P<0.05$)。与模型组比较, 手法组 MMP-13、IL-1 β 、TNF- α 和 ERK1/2 mRNA 转录水平均明显降低($P<0.05$)。⑤ 骨密度和骨小梁数目: 与空白组比较, 模型组干预后 Conn.D 和 Tb.N 均明显降低($P<0.05$), 骨小梁结构不规则、骨赘形成明显增多。与模型组比较, 手法组干预后 Conn.D

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和Tb.N均明显升高($P<0.05$),骨小梁结构更规则,骨赘形成明显减少。**结论** 筋滞骨错手法可改善KOA模型兔膝关节结构与膝关节功能,其作用机制可能与调节ERK通路抑制炎症反应和细胞外基质降解,缓解内质网应激损伤有关。

关键词 膝骨关节炎;筋滞骨错手法;膝关节功能;软骨退变;ERK信号通路

膝骨关节炎(knee osteoarthritis, KOA)是以关节退变为特征的慢性疾病,病变多累及关节软骨、滑膜、关节囊和周围软组织,临床表现为软骨结构破坏、滑膜炎反应及疼痛功能障碍,因其高发病率和致残率,严重影响患者的生活质量^[1]。KOA的发病机制复杂,涉及多种因素的相互作用,包括生物力学、遗传、炎症和骨代谢失衡等^[2],其中异常的机械应力导致关节载荷分布失衡会促进软骨细胞衰老进程^[3]。目前对KOA的临床治疗以药物镇痛、物理因子干预和关节置换手术为主,但这些方法存在一定的局限性,如非甾体抗炎药所致胃肠损伤、软骨保护剂疗效有限、手术风险高等^[4]。以“筋骨平衡”和“整体观念”为核心的中医正骨疗法在KOA治疗中具有一定优势。中医正骨手法不仅可以通过刺激穴位和经络,调节机体的免疫反应,降低关节内的炎症反应,如运用点按血海、梁丘等穴位手法,通过刺激背根神经节(dorsal root ganglia, DRG)神经元调控肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α)、白细胞介素-1 β (interleukin-1 β , IL-1 β)等炎症介质释放,使关节液炎症因子浓度下降50%以上^[5];还可以通过复位和理筋通络,调整关节的力学平衡,改善关节软骨的受力分布^[6-7]。此外,中医正骨手法能够通过调节局部血液循环和神经反射,激活Wnt/ β -catenin等骨代谢相关通路,促进成骨细胞的活性,抑制破骨细胞的异常活化,恢复骨代谢平衡,维持骨稳态^[8]。平乐“筋滞骨错”理论认为软组织力学失衡是KOA的始动因素,骨骼的病变是继发于软组织的病变^[9-10]。筋滞骨错手法具有舒筋活络、活血通经、消肿止痛的功效,在治疗骨关节疾病方面具有独特的优势^[11-13],但其作用机制尚不明确。

本研究通过筋滞骨错手法干预KOA兔模型,通过细胞外信号调节激酶(extracellular signal-regulated kinase, ERK)通路探讨其对兔KOA软骨退变及骨代谢的调控效应,为筋滞骨错手法治疗KOA提供依据。

1 材料和方法

1.1 实验动物

选用雄性健康24周龄新西兰大白兔30只,体重2.0~4.5 kg,购自郑州市兔尔康牧业有限公司,实验动物生产许可证号:SYXK(豫)2021-0015。将实验兔单笼、标准饲料、自由饮用纯净水,实验室定期紫外消毒,于河南中医药大学动物中心饲养,房间保持恒温45%~60%相对湿度。适应性喂养1周后,采用随机数字表法分为空白组和造模组,分别10、20只。本实验方案由河南中医药大学实验动物伦理审查委员会批准(审批号:IACUC-202409026)。实验过程遵循了国际兽医学编辑协会《关于动物伦理与福利的作者指南共识》和国家相关法律、法规。

1.2 主要实验仪器和试剂

1.2.1 主要仪器 低温超速离心机(德国Eppendorf公司,型号:B30154);全波长酶标仪(美国Thermo公司,型号:1681130);蛋白垂直电泳仪(美国Bio-Rad公司,型号:164-5050);蛋白转膜仪(美国Bio-Rad公司,型号:164-5050);掌上离心机(杭州茂丰仪器有限公司,型号:MF24-1021);超纯水仪(四川优普超纯科技有限公司,型号:UPR-II-10T);制冰机(斯科茨曼制冰系统有限公司,型号:XB-70);移液器[大龙兴创实验仪器(北京)有限公司];摇床(海门市其林贝尔仪器制造有限公司,型号:STS-8A-A);96孔板离心机(德国Eppendorf公司,型号:5810R);CFX96实时定量荧光PCR仪(美国Bio-Rad公司,型号:1852148);液氮罐(成都金凤液氮容器有限公司)。

1.2.2 主要试剂 4%多聚甲醛/通用型组织固定液(北京兰杰柯科技有限公司,批号:BL539A);0.45 μ m聚偏二氟乙烯(polyvinylidene fluoride, PVDF)膜(北京智杰方远科技有限公司,货号:IPVH00010);高效放射免疫沉淀分析缓冲液(radio immunoprecipitation assay, RIPA)组织-细胞快速裂解液(北京索莱宝科技有限公司,货号:R0010-100);Omni-ECL超灵敏化学发光检测试剂盒(上海雅酶生物医药科技有限公司,批号:SQ201)。PrimeScript™ RT reagent

Kit with gDNA Eraser (Perfect Real Time)(Takara Bio, RR047A)、TB Green® Premix Ex Taq™ II (Tli RNaseH Plus)(Takara Bio, RR820A)均购自宝生物工程(大连)有限公司。聚丙烯酰胺凝胶电泳(polyacrylamide gel electrophoresis, PAGE)试剂(上海雅酶生物医药科技有限公司,货号:PG212); β 肌动蛋白(Beta Actin, β -actin)抗体(货号:20536-1-AP)、TNF- α (货号:17590-1-AP)、山羊抗兔二抗(货号:SA00001-2)、细胞外蛋白调节激酶(extracellular-regulated kinase 1/2, ERK1/2)(货号:11257-1-AP)、基质金属蛋白酶 13(matrix metalloproteinase-13, MMP-13)(货号:18165-1-AP)均购自武汉三鹰生物技术有限公司。磷酸化细胞外调节蛋白激酶 1/2(phosphorylated extracellular regulated protein kinase 1/2, p-ERK1/2)(货号:TA1015)购自于艾比玛特医药科技(上海)有限公司。

1.3 模型制备与分组

1.3.1 空白组 正常饲养,不做任何处理。

1.3.2 造模组 造模组参照 Videman 造模方法,左后膝伸直位固定4周,制备KOA早期模型。准备纱布、脱脂棉,小动物高分子卷式夹板截取合适长度备用;将兔仰卧位人工固定在解剖台上,将兔左膝关节局部脱毛,并将兔左后肢屈曲,固定兔左后肢,注意保护皮肤,防止皮肤破损。先用脱脂棉包裹膝关节;右手抓握左后肢踝关节牵拉使膝关节处于伸直立位 0° 位置,左手持小动物高分子卷式夹板放置在膝前侧,夹板上部1/4在髌骨中点;操作者用纱布将夹板固定,塑形保持膝关节伸直中立位,踝关节背屈,外层缠绕纱布防止兔啃咬夹板。于首次固定后第28天进行膝关节勒凯斯纳功能活动指数(Lequesne Algofunctional Index, Lequesne MG)评级^[14]。Lequesne MG总分 >4 分且关节软骨退变增生明显,周边出现大量骨赘,关节间隙变窄,符合膝骨关节炎改变为造模成功。

1.4 干预方法

1.4.1 空白组、模型组 自由活动和饮食,不进行任何干预。

1.4.2 手法组 在造模成功后开始进行筋滞骨错手法治疗。

1.4.2.1 理筋滞手法 由1名操作者进行日常手法操作训练。①顺经络:采用捋顺手法,对患膝足三阳经、三阴经沿经络方向进行捋顺手法共3遍。②点按法:参照《实验针灸学》^[15]和《动物针灸学》^[16],

膝关节炎常规手法操作规范进行操作。操作时注意松解经筋的筋结点、筋聚点,避免手法用力过度集中,应做到柔和有力,从容和缓以达到舒筋止痛的效果。选取家兔患膝周围穴位:伏兔、梁丘、内外膝眼、血海、阳陵泉、阿是穴等穴位,手法频率60~80次/min,顺时针每穴点按2 min,12 min/次,1次/d,共干预21 d。

1.4.2.2 纠骨错手法 在理筋滞手法干预之后,术者左手持家兔股骨下端,右手持家兔患肢胫骨上端,左手固定,右手向前推送胫骨。1次/周,3次/疗程,共干预21 d。

1.5 观察指标

分别于干预前后评价以下指标。

1.5.1 行为学观察 采用Lequesne MG分级量表评价兔行为学与步态。Lequesne MG评分总分反映了膝关节功能障碍程度,总分越高,膝关节功能障碍越严重。

1.5.2 病理学改变 采用苏木精-伊红(hematoxylin and eosin, HE)染色法观察兔膝关节病理学改变。取样后固定好的组织样本用流水冲洗后进行脱水,然后包埋成蜡块,使用切片机器将其切成3 μ m厚石蜡切片,65 $^\circ$ C烤片4.5 h。在苏木精染液中染色3 min,自来水冲洗;分化液分化10 s,自来水冲洗,反蓝液反蓝5 s,流水冲洗;梯度乙醇脱水,再在伊红染液中染色5 min;二甲苯透明,中性树脂封固;光学显微镜观察,选取目标区域进行拍照、分析。

1.5.3 关节软骨及软骨下骨的组织结构变化 采用番红O-固绿染色法观察关节软骨及软骨下骨的组织结构变化。切片经过脱蜡、复水、自来水冲洗,用苏木素染色5~10 min,自来水冲洗,分化液30 s,自来水冲洗10 min返蓝,固绿染液染色5 min,自来水冲洗;乙酸分化液30 s,自来水冲洗;番红O染液染色8 min,无水乙醇脱水,二甲苯透明,中性树脂封片;显微镜观察,选取目标区域进行拍照、分析。

1.5.4 炎症与基质降解相关因子蛋白表达水平 采用Western blot检测ERK1/2、磷酸化激活的ERK1/2(pERK1/2)占比、MMP-13和TNF- α 的蛋白表达量。制备十二烷基硫酸钠聚丙烯酰胺凝胶电泳(sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-PAGE)凝胶,经过蛋白定量、电泳后,将免疫蛋白印记转到聚偏二氟乙烯(polyvinylidene fluoride, PVDF)膜上,分别用一抗(1:2 000)及二抗(1:3 000)对其进行孵育、检测,使用ImageJ软件进行灰度值

分析。

$$\text{pERK1/2 占比} = \frac{\text{pERK1/2 蛋白表达量}}{\text{总ERK1/2 蛋白表达量}} \times 100\%$$

1.5.5 炎症与基质降解相关因子 mRNA 转录水平检测 采用定量逆转录聚合酶链式反应(quantitative reverse transcription polymerase chain reaction, qRT-PCR)法检测 ERK1/2、MMP-13、IL-1β 和 TNF-α mRNA 转录水平。提取组织样本中总 RNA,取 2 μL RNA 检测其浓度。

1.5.5.1 反转录 去除基因组 DNA 反应,按反应数配置 Master Mix,5×g DNA Eraser Buffer 2 μL,gDNA Eraser 1 μL,Total RNA 1 μL,加 RNase Free dH₂O 使总体积为 10 μL 混匀,PCR 仪设置 42 °C 2 min,取出在冰上继续加入 RNase Free dH₂O 1 μL,PrimeScript^{RT}

Enzyme Mix I 1 μL,RT Primer Mix 4 μL,5×Prime-Script Buffer 2(for Real Time)4 μL,震荡离心 PCR 仪器选择 37 °C,15 min,85 °C,5 s,然后稀释 4 倍每孔加入 60 μL 焦碳酸二乙酯(diethyl pyrocarbonate,DEPC)水混匀,-20 °C 冰箱备用。

1.5.5.2 配体系 进行铺板检测时计算预混液体积,每孔加入 TB GreenPremix Ex Taq II (Tli RNaseH Plus) 5 μL,PCR Forward Primer (10 μmol) 0.4 μL,PCR Reverse Primer (10 μmol) 0.4 μL,DNA 模板(< 100 ng) 1 μL,DEPC 水 3.2 μL,配制时多配 3 个孔液体备用。在 PCR 扩增仪中充分反应,PCR 反应条件:95 °C 预变性 30 s,95 °C 变性 5 s,60 °C 退火 34 s,共 40 个循环。采用 2^{-ΔΔCt} 法进行数据的相对定量分析。qRT-PCR 检测目的基因引物序列见表 1。

表 1 qRT-PCR 引物序列
Table 1 Primer sequences of qRT-PCR

基因	引物序列(5'-3')	产物长度/bp
ERK1/2	F:TTGACGAGCAGCAGCGGAAG R:CTGGGCTTGTGGGAGACCTTG	145
MMP-13	F:TGCGGGAATCCTGAAGAAGAAGAATGC R:AAGTTTGCCTGTACCTCTAAGCC	94
IL-1β	F:CGATGGTCCCAATTACATGAAGAGC R:AGAGCCACAACGACTGACAAGAC	135
TNF-α	F:TAGTAGCAAACCCGCAAGTGGAG R:GCTGAAGAGAACCTGGGAGTAGATG	149
β-actin	F:CCATGGATGATGATATCGCCGC R:TCTGACCCATGCCACCATC	150

1.5.6 骨密度、骨小梁 采用 Micro CT 检测兔膝关节骨密度、骨小梁。将截取的整个膝关节用 4% 多聚甲醛固定 48 h 后,放入 Micro CT 仪器中进行扫描,评估软骨下骨结构和骨代谢,检测骨小梁连接密度(trabecular connectivity density,Conn.D)与骨小梁数量(trabecular number,Tb.N)。采用 Avatar 软件对 Venus[®] Micro CT 扫描结果进行显示和扫描重建分析。

1.6 统计学方法

采用 SPSS 25.0 统计软件进行数据分析。计量资料服从正态分布以($\bar{x} \pm s$)表示,多组间比较采用单因素方差分析,组间两两比较若方差齐则采用 LSD-*t* 检验,方差不齐时采用 Tamhane's *T2* 检验;不服从正态分布的数据以 *M*(*P*₂₅,*P*₇₅)表示,多组间比

较采用 Kruskal-Wallis *H* 检验。*P*<0.05 为差异具有统计学意义。

2 结 果

2.1 实验动物纳入情况

由于造模失败和干预期间死亡等原因,空白组死亡 2 只,造模组死亡 4 只,2 组最后分别纳入 8 只、16 只。造模组按随机数字表法分为模型组和手法组,每组 8 只。

2.2 3 组干预后 Lequesne MG 评分比较

与空白组比较,模型组和手法组干预后 Lequesne MG 评分均明显升高(*P*<0.05)。与模型组比较,手法组干预后 Lequesne MG 评分明显降低(*P*<0.05)。见表 2。

表2 3组干预后Lequesne MG评分比较[$M(P_{25}, P_{75})$] 分
Table 2 Comparison of Lequesne MG scores in three groups after intervention [$M(P_{25}, P_{75})$] Scores

组别	<i>n</i>	Lequesne MG 评分
空白组	8	0.00(0.00,0.75)
模型组	8	11.00(10.00,11.00) ¹⁾
手法组	8	4.00(4.00,4.75) ¹⁾²⁾

注:与空白组比较,1) $P<0.05$;与模型组比较,2) $P<0.05$ 。
Note: compared with the blank group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$.

2.3 3组兔软骨病理学改变

HE染色结果显示,干预结束后,空白组表层软骨平整、软骨细胞分布均匀、层次清晰,无聚集成团的软骨细胞,潮线连续无中断。软骨分为典型的

4层,表层软骨呈梭形,与关节面平行排列;中间层软骨细胞与关节面呈斜形排列;深层软骨细胞呈柱状排列,潮线下为钙化层。模型组内侧平台表面软骨不平整,局部可见软骨面的丢失,浅层软骨细胞减少,呈肥大分化,深层软骨细胞聚集成团。手法组表层软骨较模型组平整,细胞排列分布较均匀。见图1。

番红O-固绿染色结果显示,空白组股骨髁及平台软骨面平整,软骨细胞排列有序、分布均匀,潮线完整,软骨基质着色均匀。模型组平台软骨欠平整,可见明显软骨裂隙及缺损,软骨基质着色不均匀。手法组平台软骨较模型组平整,软骨基质染色较均匀。见图2。

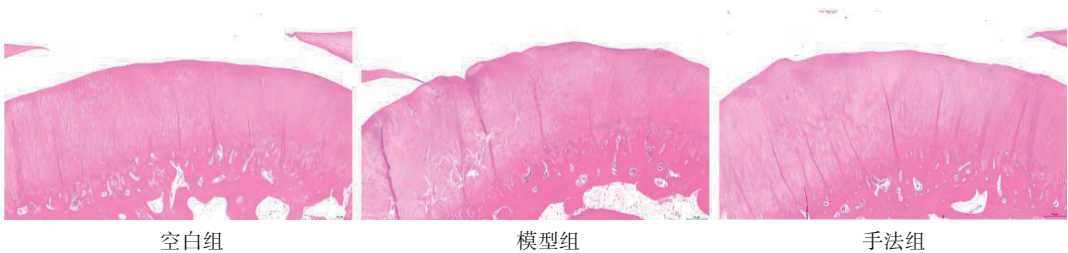


图1 3组膝关节软骨的组织形态变化HE染色(标尺200 μm)
Figure 1 HE staining of tissue morphological changes in knee joint cartilage in three groups (scale bar 200 μm)

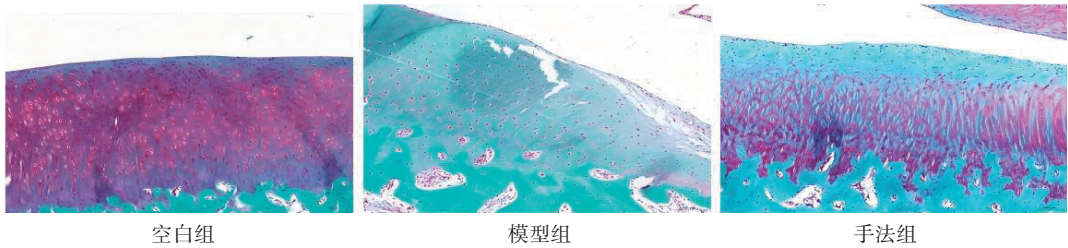


图2 3组膝关节软骨组织形态变化番红O-固绿染色(标尺100 μm)
Figure 2 Safranin O-fast green staining of tissue morphological changes in knee joint cartilage in three groups (scale bar 100 μm)

2.4 3组TNF-α、MMP-13、ERK1/2蛋白表达水平和pERK1/2占比比较

与空白组比较,模型组干预后TNF-α、MMP-13、ERK1/2蛋白表达水平和pERK1/2占比均明

显升高($P<0.05$)。与模型组比较,手法组干预后TNF-α、MMP-13、ERK1/2蛋白表达水平和pERK1/2占比均明显降低($P<0.05$)。见表3、图3。

表3 3组TNF-α、MMP-13、ERK1/2蛋白表达水平和PERK1/2占比比较($\bar{x}\pm s$)
Table 3 Comparison of protein expression levels of TNF-α, MMP-13, ERK1/2 and PERK1/2 proportion in three groups ($\bar{x}\pm s$)

组别	<i>n</i>	TNF-α	MMP-13	ERK1/2	pERK1/2占比
空白组	8	0.72±0.16	0.92±0.03	0.64±0.04	0.61±0.01
模型组	8	1.71±0.20 ¹⁾	1.28±0.04 ¹⁾	1.28±0.10 ¹⁾	1.21±0.05 ¹⁾
手法组	8	0.60±0.07 ²⁾	0.76±0.03 ²⁾	0.68±0.11 ²⁾	0.79±0.08 ²⁾

注:与空白组比较,1) $P<0.05$;与模型组比较,2) $P<0.05$ 。
Note: compared with the blank group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$.

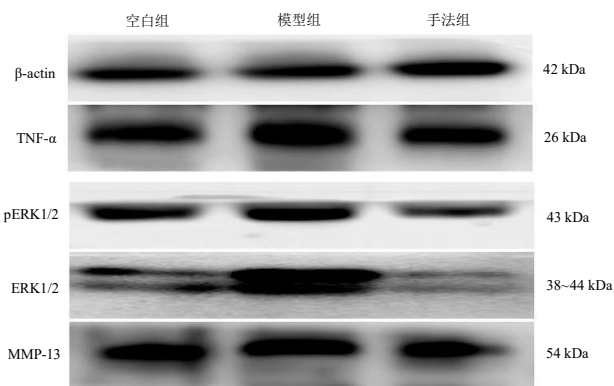


图3 3组TNF-α、MMP-13、ERK1/2和pERK1/2蛋白条带图

Figure 3 Protein band images of TNF-α, MMP-13, ERK1/2 and pERK1/2 in three groups

表4 3组TNF-α、MMP-13、IL-1β和ERK1/2 mRNA 转录水平比较($\bar{x}\pm s$)

Table 4 Comparison of mRNA transcription levels of TNF-α, MMP-13, IL-1β and ERK1/2 in three groups ($\bar{x}\pm s$)

组别	<i>n</i>	TNF-α	MMP-13	IL-1β	ERK1/2
空白组	8	0.91±0.07	0.28±0.06	0.80±0.08	0.97±0.10
模型组	8	1.21±0.07 ¹⁾	1.14±0.38 ¹⁾	1.47±0.14 ¹⁾	1.08±0.02
手法组	8	0.24±0.04 ²⁾	0.52±0.16 ²⁾	1.16±0.06 ²⁾	0.62±0.19 ²⁾

注:与空白组比较,1) $P<0.05$;与模型组比较,2) $P<0.05$ 。

Note: compared with the blank group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$.

表5 3组Conn.D和Tb.N比较($\bar{x}\pm s$)

Table 5 Comparison of Conn.D and Tb.N in three groups ($\bar{x}\pm s$)

组别	<i>n</i>	Conn.D	Tb.N
空白组	8	4.22±0.18	1.75±0.05
模型组	8	1.55±0.15 ¹⁾	0.94±0.02 ¹⁾
手法组	8	3.14±0.08 ²⁾	1.49±0.15 ²⁾

注:与空白组比较,1) $P<0.05$;与模型组比较,2) $P<0.05$ 。

Note: compared with the blank group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$.

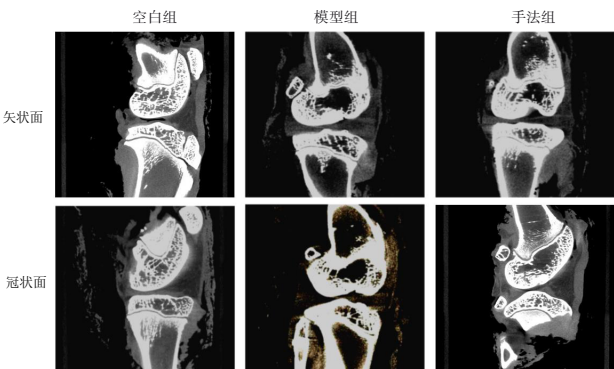


图4 3组Micro CT形态图

Figure 4 Morphological images of Micro CT in three groups

2.5 3组MMP-13、IL-1β、TNF-α和ERK1/2 mRNA 转录水平比较

与空白组比较,模型组干预后TNF-α、MMP-13和IL-1β mRNA 转录水平均明显升高($P<0.05$)。与模型组比较,手法组MMP-13、IL-1β、TNF-α和ERK1/2 mRNA 转录水平均明显降低($P<0.05$)。见表4。

2.6 3组骨密度和骨小梁数目比较

与空白组比较,模型组干预后Conn.D和Tb.N均明显降低($P<0.05$),骨小梁结构不规则、骨赘形成明显增多。与模型组比较,手法组干预后Conn.D和Tb.N均明显升高($P<0.05$),骨小梁结构更规则,骨赘形成明显减少。见表5、图4。

3 讨 论

3.1 筋滞骨错手法可改善KOA模型兔膝关节结构与膝关节功能

本研究结果显示,与模型组比较,手法组干预后Lequesne MG评分明显降低,Conn.D和Tb.N均明显升高,骨小梁结构更规则,骨赘形成明显减少。这提示,筋滞骨错手法可改善KOA模型兔膝关节结构与膝关节功能。可能与以下因素有关:①筋滞骨错手法可纠正筋骨整体架构。KOA在中医属“痹证”范畴,病机关键在肝肾不足,筋脉失养,风寒湿邪入侵,痹阻经络,发展成为“筋出槽,骨错缝”的筋骨失衡状态。筋滞骨错手法依照筋骨构架的关系,纠正“脊柱-骨盆-关节”的整体力学构线和筋骨整体构架,着重调整关节的结构和功能,通过筋滞骨错手法松解筋膜、调整关节位置、恢复关节活动度,维持膝关节和机体整体的平衡和稳定,局部与整体相结合,达到治疗KOA的目的^[17-18]。②筋滞骨错手法可调整整体力学结构。筋滞骨错手法的特色在于其整体观和力学观,不仅仅是关注膝关节本身,更关注脊柱、骨盆与膝关节的联动关系,通过调

整整体力学结构以改善局部问题^[19-20]。其优势在于能够从根本上改善膝关节的受力情况,减轻疼痛,恢复功能。这与靳博等^[21]研究结果相似。③筋滞骨错手法可促进骨结构的修复与重建。骨小梁数目减少反映了骨量的丢失,是KOA进展的重要特征,表明骨代谢失衡的加剧^[22]。从“筋骨同治”角度,筋滞骨错手法通过力学调整改善关节整体状态,在改善关节功能障碍的同时,还能促进骨结构的修复与重建。

3.2 筋滞骨错手法改善KOA模型兔膝关节功能可能与调节ERK通路有关

本研究结果显示,与模型组比较,手法组干预后TNF- α 、MMP-13、ERK1/2蛋白表达水平和pERK1/2占比均明显降低,MMP-13、IL-1 β 、TNF- α 和ERK1/2 mRNA转录水平均明显降低,这提示筋滞骨错手法改善KOA模型兔膝关节功能可能与调节ERK通路有关。这与YAMAMOTO等^[23-24]研究结果相似。可能与以下因素有关:①抑制炎症反应和细胞外基质降解。炎症反应与细胞外基质降解是KOA软骨退变的核心环节。KOA模型兔膝关节组织中促炎因子TNF- α 及软骨降解关键酶MMP-13蛋白表达均明显升高。TNF- α 不仅可能直接损伤软骨细胞,还可能促进MMP-13的表达和活性,两者协同作用加速软骨破坏^[25],刺激破骨细胞活性、降低成骨细胞活性,最终导致骨量减少^[26]。MMP-13作为特异性降解二型胶原的蛋白酶,其过度表达是软骨基质崩解的重要标志。筋滞骨错手法干预不仅可纠正力学失衡,减轻异常应力对滑膜及软骨细胞的机械性刺激;还可以改善局部血运与组织液循环,促进炎性物质清除。该手法间接抑制TNF- α 等核心炎症因子的合成与释放,阻断其对MMP-13等降解酶的诱导作用,延缓软骨退变进程。②缓解内质网应激损伤。ERK通路可被机械应力、炎症因子(如IL-1 β 、TNF- α)等多种刺激激活,在炎症、软骨降解和疼痛等方面起着促进作用^[27-28]。激活的ERK可能会促进软骨细胞凋亡,抑制软骨细胞增殖,并促进MMP-13等降解酶的表达,加速软骨的降解,促进促炎因子TNF- α 、IL-1 β 产生,募集炎症细胞到关节腔,加重炎症反应和骨代谢紊乱^[29-31]。此外,激活的ERK通过磷酸化转录因子,上调MMP-13等基因表达,抑制软骨细胞基质合成^[32],并促进炎症介质的释放,导致软骨修复能力下降^[33]。筋滞骨错手法可能通过纠正软组织力学失衡,减轻异常压力,调控

ERK活性,减少神经源性炎症,保护软骨细胞,并抑制MMP-13表达和pERK1/2活化,间接缓解内质网应激损伤^[34-35],减少软骨细胞凋亡,并促进膝关节功能恢复。这与RELLMANN等^[36-38]研究结果相似。

4 小 结

筋滞骨错手法可有效改善KOA模型兔膝关节结构与膝关节功能,其机制可能与调节ERK信号通路、抑制炎症反应和内皮质应激损伤有关。这为筋滞骨错手法治疗KOA提供了理论基础。但对于已经严重损伤的软骨,特别是关节面破坏较为严重的情况,筋滞骨错手法可能仅可有效减轻KOA症状,延缓病情发展,对软骨的直接修复效果可能有限。未来研究还需进一步探究筋滞骨错手法对不同类型、严重程度KOA的治疗效果,并深入探讨其作用机制,为筋滞骨错手法的临床治疗提供更多的循证依据。

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Effects and Mechanisms of Sinew Stagnation and Bone Dislocation Manipulation on Cartilage Degeneration in Rabbit Knee Osteoarthritis via the Extracellular Signal-Regulated Kinase Pathway

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ABSTRACT Objective To investigate the effects of the sinew stagnation and bone dislocation manipulation on cartilage degeneration in rabbits with knee osteoarthritis (KOA) via the extracellular signal-regulated kinase (ERK) pathway and to elucidate its underlying mechanisms. **Methods** A total of 30 healthy male New Zealand White rabbits were randomly divided into blank group ($n=10$), and modeling group ($n=20$). The blank group did not received treatment, whereas the modeling group adopted the Videman modeling method to establish an early KOA model. Due to modeling failure and death during the intervention period, 8 and 16 cases were ultimately included in the blank group and the modeling group, respectively. The modeling group was further divided into model group and manipulation group, with eight cases in each group. The blank group and the model group were permitted free activity and diet without any intervention. The manipulation group received sinew stagnation and bone dislocation manipulation technique after successful modeling, including manipulation of regulating tendon stagnation (manipulation along the three Yang meridians of the foot and three Yin meridians of the foot; pressing acupoints such as Futu (ST32), Liangqiu (ST34), medial and lateral Xiyan (EX-LE4, EX-LE5), Xuehai (SP10), Yanglingquan (G34), Ashi acupoints, at a rate of 60-80 times per minute, for two minutes per acupoint, 12 minutes per session, once a day, for a total of 21 days, and the bone dislocation correcting technique once a week, three times a course, with a total intervention of 21 days. The Lequesne Algofunctional Index (Lequesne MG score) was used to assess rabbit behavior and gait. Hematoxylin-Eosin (HE) staining was used to observe pathological changes in rabbit knee joints. Safranin O-Fast Green staining was used to evaluate structural alterations in articular cartilage and subchondral bone. Western blot analysis was carried out to determine the protein expression levels of ERK1/2, phosphorylated ERK1/2 (pERK1/2), matrix metalloproteinase-13 (MMP-13), and tumor necrosis factor- α (TNF- α). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was applied to measure the mRNA transcription levels of ERK1/2, MMP13, interleukin-1 beta (IL-1 β), and TNF- α . Micro-CT was utilized to assess trabecular connectivity density (Conn.D) and trabecular number (Tb.N) in rabbit knee joints. **Results** (1) Lequesne MG score: compared with the blank group, the Lequesne MG scores of the model group and the manipulation group increased significantly after intervention ($P<0.05$). Compared with the model group, the Lequesne MG score in the manipulation group decreased significantly after intervention ($P<0.05$). (2) Cartilage pathological changes: the cartilage surface of the medial plateau was uneven in the model group, with local loss of cartilage. The superficial chondrocytes showed a decrease in number and hypertrophic differentia-

tion, while the deep chondrocytes were clustered. Obvious cartilage fissures or defects were observed, and cartilage matrix staining was uneven. The superficial cartilage in the manipulation group was more regular compared to that of the model group. The arrangement and distribution of chondrocytes were more uniform, and cartilage matrix staining was more even. (3) Expression levels of TNF- α , MMP-13, ERK1/2 protein and pERK1/2 proportion: compared with the blank group, the expression levels of TNF- α , MMP-13, ERK1/2 protein, as well as the proportion of pERK1/2 in the model group increased significantly after intervention ($P<0.05$). Compared with the model group, the expression levels of TNF- α , MMP-13, ERK1/2 protein, as well as the proportion of pERK1/2 in the manipulation group decreased significantly after intervention ($P<0.05$). (4) The mRNA transcription level of MMP-13, IL-1 β , TNF- α , and ERK1/2: compared with the blank group, the mRNA transcription level of TNF- α , MMP13, and IL-1 β in the model group increased significantly after intervention ($P<0.05$). Compared with the model group, mRNA transcription levels of MMP-13, IL-1 β , TNF- α , and ERK1/2 in the manipulation group decreased significantly after intervention ($P<0.05$). (5) Bone mineral density and trabecular number: compared with the blank group, the values of Conn.D and Tb.N in the model group decreased significantly after intervention ($P<0.05$); the trabecular structure was irregular, and the osteophyte formation increased significantly. Compared with the model group, the values of Conn.D and Tb.N in the manipulation group increased significantly after intervention ($P<0.05$), the trabecular structure was more regular, and the osteophyte formation decreased significantly. **Conclusion** The sinew stagnation and bone dislocation manipulation technique can improve the knee joint structure and knee joint function of KOA model rabbits, and its mechanism may be related to regulating the ERK pathway to inhibit inflammatory responses and extracellular matrix degradation, and alleviate endoplasmic reticulum stress damage.

KEY WORDS knee osteoarthritis; sinew stagnation and bone dislocation manipulation technique; knee joint function; cartilage degeneration; ERK signaling pathway

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Application of Transcutaneous Auricular Vagus Nerve Stimulation in Motor Rehabilitation after Stroke

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ABSTRACT Transcutaneous auricular vagus nerve stimulation (taVNS) activates neural pathways akin to invasive vagus nerve stimulation (iVNS). With the advantages of low cost, safety, and high tolerability, taVNS serves as an alternative neuromodulatory technique to iVNS and presents promising prospects for motor function rehabilitation after stroke. However, there is a lack of consensus on specific application parameters and therapeutic effects of taVNS, with the underlying mechanisms remaining to be elucidated. This review comprehensively examines the application of taVNS in post-stroke motor rehabilitation, encompassing both asynchronous and synchronous combination with upper limb training, as well as monotherapy with taVNS. Its mechanisms of action include anti-inflammatory and neuroprotective effects, enhancement of blood-brain barrier integrity, and modulation of cortical excitability and neuroplasticity. The review further analyzed the stimulation parameters, safety, and efficacy of taVNS. However, high-quality clinical studies are still insufficient. The majority of taVNS research has focused on limb function scores without exploring the underlying neurophysiological mechanisms. There is no consensus on taVNS stimulation parameters, including stimulation intensity, frequency, duration, and timing. The safety profile of taVNS applied to the right ear warrants further validation. Limited studies have reported lateralized effects when taVNS is applied to the left ear. Future large-sample and high-quality studies are required to establish optimal stimulation parameters and elucidate the mechanisms of action, thereby informing the development of the most effective taVNS treatment protocols for clinical practice.

KEY WORDS stroke; transcutaneous auricular vagus nerve stimulation; neuromodulatory technique; motor function rehabilitation

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