·基础研究·

舒尼替尼干预大鼠膝骨关节炎的实验研究

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摘 要 **目的**：探讨舒尼替尼对大鼠膝骨关节炎（knee osteoarthritis，KOA）的影响。**方法**：选取 32 只雄性 SD 大鼠，随机分为空白组、模型组、4 周治疗组及 8 周治疗组，每组 8 只。除空白组外，其余 3 组大鼠均采用木瓜蛋白酶进行 KOA 造模。造模完成后，空白组大鼠正常喂养；4 周治疗组和 8 周治疗组大鼠按照 1 mg·kg^{-1}以舒尼替尼灌胃，每天 1 次，2 组分别持续干预 4 周和 8 周；模型组大鼠每天以同等体积生理盐水灌胃。药物干预结束后处死各组大鼠，分别进行膝关节 MRI 检查、关节液血管内皮生长因子（vascular endothelial growth factor，VEGF）和基质金属蛋白酶（matrix metalloproteinase，MMP）－13 水平测定、膝关节组织病理学观察。**结果**：①膝关节 MRI 检查结果。大鼠膝关节矢状位 MRI 显示，空白组大鼠膝关节结构完整，关节表面有完整的软骨，表面光滑，软骨与骨组织连接紧密，软骨下无炎症及囊性病变，软骨下骨无磨损、无骨赘；模型组大鼠膝关节结构明显破坏，表面软骨明显磨损，特别是胫骨平台有明显软骨及软骨下骨破损，软骨下有囊性病变；4 周治疗组大鼠膝关节结构基本完整，软骨略粗糙，但胫骨平台下仍见软骨下炎症及软骨下骨破损，软骨下存在囊性病变；8 周治疗组大鼠膝关节结构完整，软骨完整光滑，软骨下骨无明显破损，软骨下无明显囊性病变，但软骨下仍有一定程度的炎症。②膝关节液 VEGF、MMP－13 水平测定结果。4 组大鼠关节液 VEGF 水平比较，差异有统计学意义［（152.26±31.17）pg·mL^{-1}，（237.95±69.10）pg·mL^{-1}，（168.43±47.47）pg·mL^{-1}，（136.75±27.48）pg·mL^{-1}，$F=14.575$，$P=0.000$］。模型组关节液 VEGF 水平高于空白组、4 周治疗组和 8 周治疗组（$P=0.000$，$P=0.000$，$P=0.000$）；空白组与 4 周治疗组、8 周治疗组比较，差异均无统计学意义（$P=0.332$，$P=0.181$）；4 周治疗组与 8 周治疗组比较，差异无统计学意义（$P=0.060$）。4 组大鼠关节液 MMP－13 水平比较，差异有统计学意义［（60.85±13.70）pg·mL^{-1}，（94.98±37.63）pg·mL^{-1}，（75.43±20.23）pg·mL^{-1}，（68.54±19.82）pg·mL^{-1}，$F=5.686$，$P=0.002$］。模型组关节液 MMP－13 水平高于空白组、4 周治疗组和 8 周治疗组（$P=0.028$，$P=0.003$，$P=0.000$）；空白组与 4 周治疗组、8 周治疗组比较，差异均无统计学意义（$P=0.098$，$P=0.242$）；4 周治疗组与 8 周治疗组比较，差异无统计学意义（$P=0.431$）。③膝关节组织病理学观察结果。空白组大鼠膝关节整体结构完整，软骨面光滑，无坏死、溃疡，骨小梁结构、形态完整；模型组大鼠膝关节有典型的软骨受损表现，软骨面不光滑，部分软骨脱失，软骨细胞排列紊乱，骨小梁不完整；4 周治疗组大鼠膝关节面较模型组略光滑，软骨细胞排列尚整齐、分布均匀，骨小梁的结构、形态较模型组完整，但仍有部分碎裂；8 周治疗组大鼠膝关节软骨光滑，软骨细胞分布均匀、排列整齐，各层次清晰，无明显细胞簇集现象，潮线完整，骨小梁结构基本完整。**结论**：舒尼替尼能通过抑制 KOA 大鼠膝关节 VEGF 的表达，抑制 MMP－13 的表达，从而延缓膝关节退变，其效果与作用时间有关。

关键词 骨关节炎，膝；血管内皮生长因子类；基质金属蛋白酶 13；舒尼替尼；动物实验

An experimental study of sunitinib for prevention of knee osteoarthritis in rats

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ABSTRACT **Objective**：To explore the effect of sunitinib on knee osteoarthritis（KOA）in rats. **Methods**：Thirty－two male SD rats were selected and were randomly divided into blank group，model group，4－week treatment group and 8－week treatment group，8 cases in each group. The KOA rat models were created in model group，4－week treatment group and 8－week treatment group by using papain，while the rats in blank group were not given any intervention. After the modeling，the rats in blank group were fed normally，and the rats in 4－week treatment group and 8－week treatment group were intragastric administrated with sunitinib（1 mg/kg），once a day for consecutive 4 and 8 weeks respectively；while the rats in model group were intragastric administrated with the same dose of normal saline everyday. After the end of drug intervention，the rats in each group were executed. MRI examination and histopathological observation on knee joint and determination of

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knee joint fluid level of vascular endothelial growth factor(VEGF) and matrix metalloproteinase(MMP) -13 were performed on rats in each group. **Results**: The results of sagittal MRI examination of knee joint showed that in blank group(1) the structure of knee - joint was complete;(2) the articular surface was smooth and the articular cartilage was intact;(3) the cartilage tissue was closely connected with bone tissue;(4) no inflammation and cystis degeneration were found under cartilage;(5) no abrasion and osteophyte were found in subchondral bone. The sagittal MRI images also showed that in model group(1) the structure of knee - joint was obviously damaged;(2) the superne cartilage was obviously worn;(3) obvious damages of cartilage and subchondral bone and obvious subchondral cystis degeneration were found under the tibial plateau. In 4 - week treatment group, the structure of knee - joint was basically intact and the cartilage was roughish, while subchondral inflammation, bone damages and cystis degeneration were found under the tibial plateau. In 8 - week treatment group, the structure of knee - joint was intact and the cartilage was complete and smooth, and no obvious subchondral bone damages and subchondral cystis degeneration were found, while some subchondral inflammation was found. There was statistical difference in the level of VEGF in knee joint fluid between the 4 groups (152.26 +/- 31.17, 237.95 +/- 69.10, 168.43 +/- 47.47, 136.75 +/- 27.48 pg/mL, $F = 14.575$, $P = 0.000$). The level of VEGF in knee joint fluid was higher in model group compared to blank group, 4 - week treatment group and 8 - week treatment group ($P = 0.000$, $P = 0.000$, $P = 0.000$); and there was no statistical difference in the level of VEGF between blank group and 4 - week treatment group and between blank group and 8 - week treatment group ($P = 0.332$, $P = 0.181$); and there was no statistical difference in the level of VEGF between 4 - week treatment group and 8 - week treatment group ($P = 0.060$). There was statistical difference in the level of MMP - 13 in knee joint fluid between the 4 groups (60.85 +/- 13.70, 94.98 +/- 37.63, 75.43 +/- 20.23, 68.54 +/- 19.82 pg/mL, $F = 5.686$, $P = 0.002$). The level of MMP - 13 in knee joint fluid was higher in model group compared to blank group, 4 - week treatment group and 8 - week treatment group ($P = 0.028$, $P = 0.003$, $P = 0.000$); and there was no statistical difference in the level of MMP - 13 between blank group and 4 - week treatment group and between blank group and 8 - week treatment group ($P = 0.098$, $P = 0.242$); and there was no statistical difference in the level of MMP - 13 between 4 - week treatment group and 8 - week treatment group ($P = 0.431$). The results of histopathological observation on knee joints of rats in blank group showed that(1) the integral structure of the knee joint was complete;(2) the surface of cartilage was smooth without necrosis and ulcer;(3) the structure and morphology of the trabecular bone were complete. The results of histopathological observation on knee joints of rats in model group showed that(1) the knee joint was characterized by typical cartilaginous damage;(2) the surface of cartilage was unsmooth and some of the cartilages were lost;(3) the chondrocytes were disorganized;(4) the trabecular bone was incomplete. In 4 - week treatment group, the knee joint surface was slightly smoother compared to model group, and the chondrocytes were regularly arranged and uniformly distributed, and the structure and morphology of the trabecular bone were more complete compared to model group, while some fragments of trabecular bone were still found. In 8 - week treatment group, the knee articular cartilage was smooth and the chondrocytes uniformly distributed and regularly arranged with clear layers; no chondrocytes clustered together, and the tidal line was complete and the structure of trabecular bone was basically complete. **Conclusion**: Sunitinib can inhibit the expression of MMP - 13 through inhibiting the expression of VEGF in knee joints of KOA rat models, which may be the mechanisms of action for delaying knee joint degeneration, and its effect is related to the action time.

Key words　osteoarthritis, knee; vascular endothelial growth factors; matrix metalloproteinase 13; sunitinib; animal experimentation

膝骨关节炎(knee osteoarthritis, KOA)是临床的常见病、多发病,已逐渐成为中老年人主要的致残因素之一[1]。血管内皮生长因子(vascular endothelial growth factor, VEGF)在 KOA 的进展中起着重要作用,而且 VEGF 也已被证实能影响软骨细胞增殖、凋亡和代谢,刺激基质金属蛋白酶类(matrix metalloproteinases, MMPs)及其他代谢介质释放,降解软骨基质[2]。舒尼替尼是一种多靶点的小分子酪氨酸激酶抑制剂,主要作用于 VEGF 家族受体,已被广泛应用于肾细胞癌、肝癌的治疗中[3]。本研究拟通过探讨舒尼替尼干预对大鼠 KOA 的影响,为 KOA 的临床治疗提供新的思路。

1　材料与仪器

1.1　实验动物　雄性健康清洁级 SD 大鼠 32 只,体质量(190 ± 10)g,由浙江中医药大学实验动物中心提供,实验动物合格证号 SCXK(沪):2012 - 0002。实验方案通过医学动物实验伦理委员会批准。

1.2　试剂与仪器　舒尼替尼(MCE 公司,货号 HY - 10255A,规格 200 mg),木瓜蛋白酶(Sigma,货号 P3250 - 25g,规格 25 g),大鼠 VEGF ELISA 试剂盒(武汉新启迪生物科技有限公司,批号 34289180),大鼠 MMP - 13 ELISA 试剂盒(武汉新启迪生物科技有

限公司,批号 38323203),HPX－9052 MBE 数显不锈钢电热培养箱(上海博讯实业有限公司医疗设备厂),DNM－9602 酶标分析仪(北京普朗新技术有限公司),Signa 3.0 T 磁共振成像仪(GE 公司)。

2　方　法

2.1　分组及造模　将 32 只大鼠随机分为空白组、模型组、4 周治疗组及 8 周治疗组,每组 8 只。各组大鼠均在相同条件下笼养,自由摄食、饮水,室温 18～22 ℃。除空白组外,其余 3 组大鼠均进行 KOA 造模。采用呼吸麻醉机进行麻醉,麻醉后将大鼠仰卧位固定于木板上,剃去左右后肢膝关节周边 1 cm 区域的毛发,络合碘消毒后再以体积分数 75% 的乙醇脱碘。膝关节屈曲 45°,以髌骨下极髌腱外缘为进针点,向髁间窝方向进针,针尖到达股骨髁后回撤 2 mm,注入 4% 木瓜蛋白酶溶液(以木瓜蛋白酶和生理盐水配制)0.2 mL,共注射 3 次,每次间隔 2 d。2 周后即可建立大鼠 KOA 模型[4]。

2.2　药物干预　造模完成后,空白组大鼠正常喂养;4 周治疗组和 8 周治疗组大鼠按照 1 mg · kg^{-1} 以舒尼替尼灌胃,每天 1 次,2 组分别持续干预 4 周和 8 周;模型组大鼠每天以同等体积生理盐水灌胃。

2.3　实验指标观察　药物干预结束后脱颈处死各组大鼠。分别进行膝关节 MRI 检查、关节液 VEGF 和 MMP－13 水平测定、膝关节组织病理学观察。

2.3.1　膝关节 MRI 检查　采用 GE Signa 3.0 T 磁共振成像仪对各组大鼠膝关节进行扫描。矢状位三维抑脂扰相梯度回波序列扫描,TR/TE:580 ms/14 ms,视野 180 mm×180 mm,矩阵 256×25,激励 2 次。

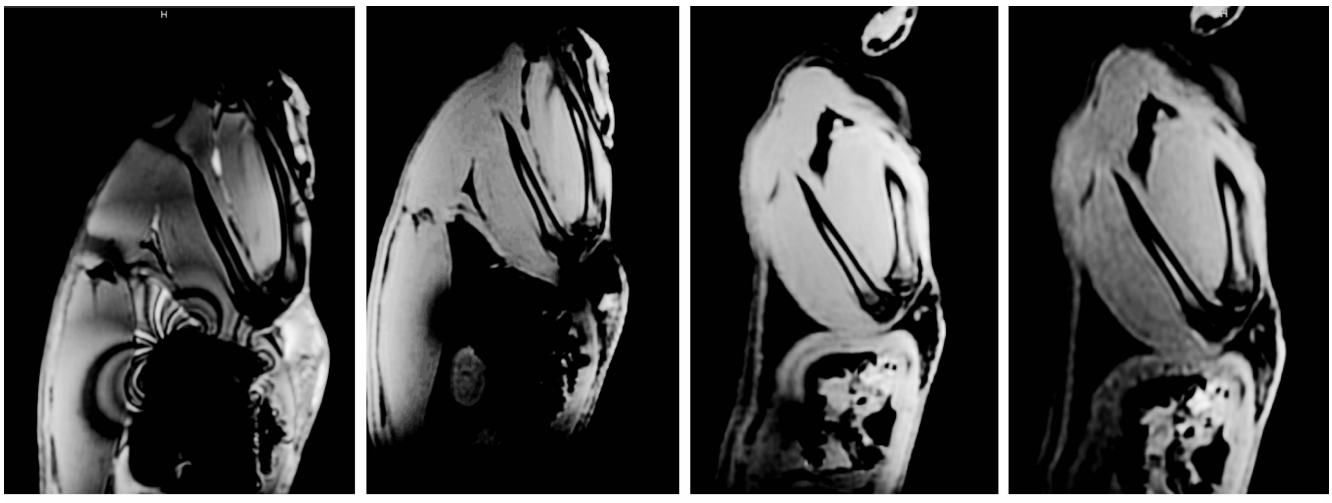
2.3.2　关节液 VEGF 和 MMP－13 水平测定　切开双侧膝关节腔,用 1 mL 生理盐水冲洗关节腔,收集冲洗液作为关节液标本。4000 r · min^{-1} 离心 5 min(离心半径 6 cm),提取上清液,置于－20 ℃冰箱密封保存。所有标本收集结束后应用 ELISA 法检测关节液中 VEGF、MMP－13 的水平。

2.3.3　膝关节组织病理学观察　在距离股骨内外髁连线以上 3 cm 处截断股骨、距离胫骨平台下方 3 cm 处切断胫骨,作为大鼠膝关节标本,置于 10% 甲醛溶液中固定 48 h。进行脱钙、水化、常规 HE 染色、封片处理后,在光学显微镜下观察软骨面、软骨下骨和骨小梁结构,选择合适视野拍照并保存。

2.4　数据统计分析　采用 SPSS19.0 软件进行数据统计学分析。4 组大鼠关节液 VEGF 水平、MMP－13 水平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　膝关节 MRI 检查结果　大鼠膝关节矢状位 MRI 显示,空白组大鼠膝关节结构完整,关节表面有完整的软骨,表面光滑,软骨与骨组织连接紧密,软骨下无炎症及囊性病变,软骨下骨无磨损、无骨赘[图 1(1)];模型组大鼠膝关节结构明显破坏,表面软骨明显磨损,特别是胫骨平台有明显软骨及软骨下骨破损,软骨下有囊性病变[图 1(2)];4 周治疗组大鼠膝关节结构基本完整,软骨略粗糙,但胫骨平台下仍见软骨下炎症及软骨下骨破损,软骨下存在囊性病变[图 1(3)];8 周治疗组大鼠膝关节结构完整,软骨完整光滑,软骨下骨无明显破损,软骨下无明显囊性病变,但软骨下仍有一定程度的炎症[图 1(4)]。



(1)空白组　(2)模型组　(3)4周治疗组　(4)8周治疗组

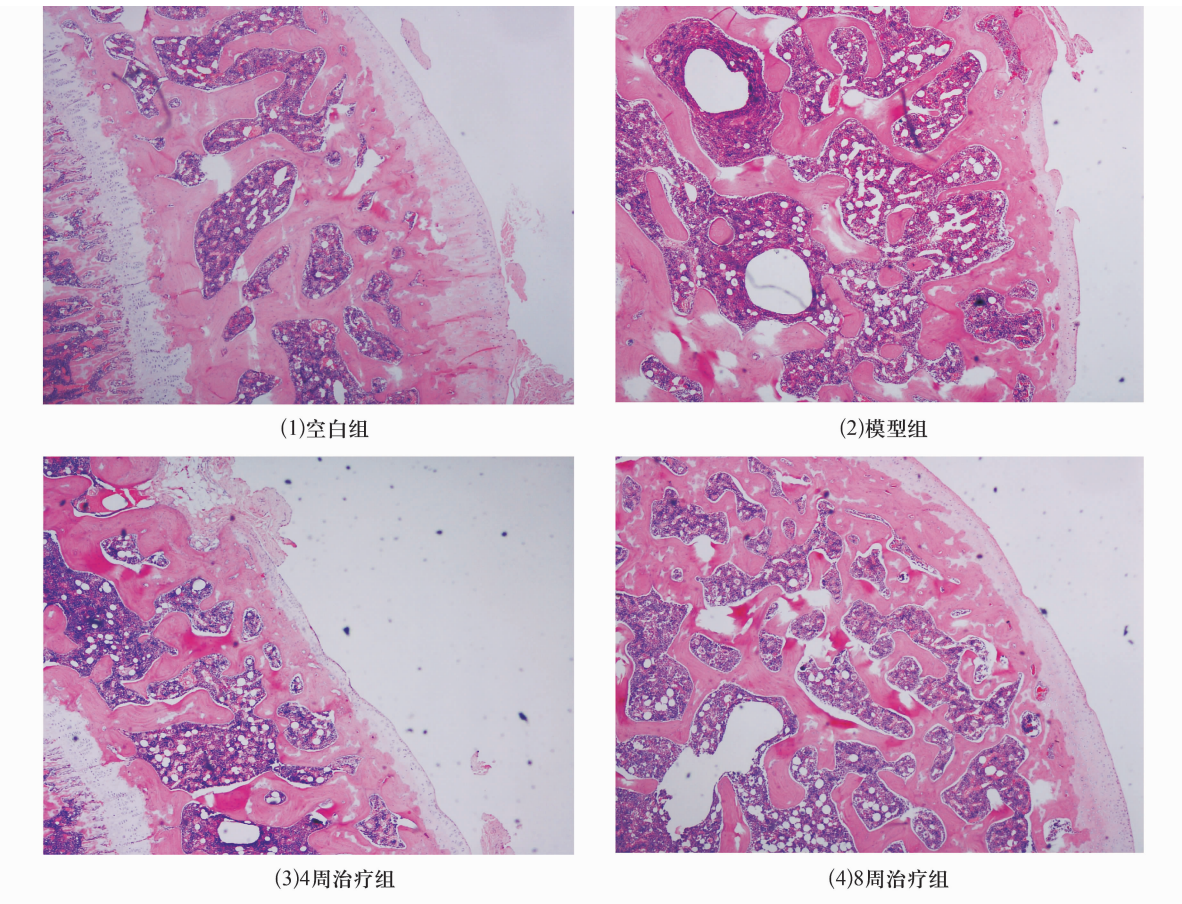
图 1　4 组大鼠膝关节矢状位 MRI 检查结果

3.2　膝关节液 VEGF、MMP－13 水平测定结果　4 组大鼠关节液 VEGF 水平比较，差异有统计学意义。模型组关节液 VEGF 水平高于空白组、4 周治疗组和 8 周治疗组（$P=0.000$，$P=0.000$，$P=0.000$）；空白组与 4 周治疗组、8 周治疗组比较，差异均无统计学意义（$P=0.332$，$P=0.181$）；4 周治疗组与 8 周治疗组比较，差异无统计学意义（$P=0.060$）。4 组大鼠关节液 MMP－13 水平比较，差异有统计学意义。模型组关节液 MMP－13 水平高于空白组、4 周治疗组和 8 周治疗组（$P=0.028$，$P=0.003$，$P=0.000$）；空白组与 4 周治疗组、8 周治疗组比较，差异均无统计学意义（$P=0.098$，$P=0.242$）；4 周治疗组与 8 周治疗组比较，差异无统计学意义（$P=0.431$）。见表 1。

3.3　膝关节组织病理学观察结果　空白组大鼠膝关节整体结构完整，软骨面光滑，无坏死、溃疡，骨小梁结构、形态完整[图 2(1)]；模型组大鼠膝关节有典型的软骨受损表现，软骨面不光滑，部分软骨脱失，软骨细胞排列紊乱，骨小梁不完整[图 2(2)]；4 周治疗组大鼠膝关节面较模型组略光滑，软骨细胞排列尚整齐、分布均匀，骨小梁的结构、形态较模型组完整，但仍有部分碎裂[图 2(3)]；8 周治疗组大鼠膝关节软骨光滑，软骨细胞分布均匀、排列整齐，各层次清晰，无明显细胞簇集现象，潮线完整，骨小梁结构基本完整[图 2(4)]。

表 1　4 组大鼠膝关节液血管内皮生长因子及基质金属蛋白酶－13 水平　$\bar{x}\pm s$，pg·mL^{-1}

组别	样本量(只)	血管内皮生长因子	基质金属蛋白酶－13
空白组	8	152.26±31.17	60.85±13.70
模型组	8	237.95±69.10	94.98±37.63
4 周治疗组	8	168.43±47.47	75.43±20.23
8 周治疗组	8	136.75±27.48	68.54±19.82
F 值		14.575	5.686
P 值		0.000	0.002



(1)空白组　(2)模型组　(3)4周治疗组　(4)8周治疗组

图 2　4 组大鼠膝关节组织病理学观察结果(HE 染色　×40)

4 讨 论

KOA 的主要病理表现为软骨、软骨下骨的改变及骨质增生[5]。VEGF 也称为血管通透性因子,具有促进血管生成,增加血管通透性的作用,是 KOA 发展过程中导致软骨下血管新生、滑膜炎形成的重要炎性递质[6]。VEGF 能通过激活内皮细胞,使小血管基膜、内皮基质在 MMPs 的作用下降解,并且通过促进血管内皮细胞增殖、分化及迁移,促进软骨下骨及滑膜的血管新生,增加血管的通透性[7]。OA 血管生成不仅可导致炎症,而且能通过新生血管转运炎性因子,致使炎性反应得以持续[8-9],同时还能阻断软骨和滑液接触,影响软骨的营养供应,导致软骨降解和骨质破坏[10]。此外,血管新生还可直接刺激破骨细胞前体及破骨细胞,导致关节骨质破坏[11]。

贾奇学等[12]用白介素 -1β 诱导大鼠关节软骨细胞,模拟 OA 软骨细胞破坏的病理学改变,发现 VEGF 具有上调软骨细胞 MMP -13 表达的作用,二者在 OA 的进程中高度相关,一方面能促进炎症反应,另一方面可加快软骨破坏[13]。在 VEGF 的作用下,MMP -13 的 mRNA 及蛋白表达水平显著升高,而 MMP -13 作为已知效应最强的Ⅱ型胶原纤维降解酶[14],其表达的加强与软骨结构的破坏呈正相关,在 KOA 的发病进展中起着重要作用。

本研究的结果提示,舒尼替尼能通过抑制 KOA 大鼠膝关节 VEGF 的表达,抑制 MMP -13 的表达,从而延缓膝关节退变,其效果与作用时间有关。

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