痛风性关节炎湿热证病证结合模型的建立

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摘　要　目的：探讨建立痛风性关节炎湿热证病证结合大鼠模型的方法。**方法**：将 40 只成年健康雄性 SD 大鼠随机分为 4 组，每组 10 只，体质量（200 ± 20）g。A、B 组正常喂养，B 组于造模开始后第 13 天在右侧踝关节腔注射微晶尿酸钠溶液。C、D 组正常喂养，同时自由饮用 200 g · L^{-1}蜂蜜水，并分别以油脂（1 g · 100g^{-1}）或 52% 红星二锅头酒（1 mL · 100 g^{-1}）灌胃，二者交替进行，持续 10 d。自第 11 天开始，置入温度（32 ± 2）℃、相对湿度（92 ± 3）% 的人工气候箱中喂养。D 组大鼠同时于第 13 天在右侧踝关节腔注射微晶尿酸钠溶液。造模期间观察大鼠的饮食、精神状态、大小便、舌象等情况；造模开始后第 15 天，测定尿液水通道蛋白 2 含量；造模开始后第 16 天，测定血浆内皮素和血浆降钙素基因相关肽含量；造模结束后处死大鼠，观察舌组织和右侧踝关节滑膜组织的病理变化。**结果**：①一般情况观察结果。A 组大鼠喂养期间，饮食、饮水、活动及大便均正常。B 组大鼠造模前期饮食、饮水、活动及大便均正常，自第 13 天开始饮食、饮水减少，烦躁不安，右踝关节明显增粗，且红、肿、热，伴活动受限。C 组大鼠开始造模后逐渐出现饮食、饮水量减少，嗜卧懒动、行动呆滞，毛发蓬松，颜色枯槁，小便黄；造模第 5 天，开始出现大便溏泻或粘腻；第 11 天出现明显呼吸粗重，烦躁不安，毛发疏松、粗糙，阴囊松弛下垂，大便溏泻或粘腻症状加重，渐见肛周污秽。D 组大鼠造模后症状与 C 组相同，第 13 天开始合并出现 B 组造模后的相同表现。②尿液水通道蛋白 2 含量测定结果。4 组大鼠尿液水通道蛋白 2 含量比较，差异有统计学意义［（0.251 ± 0.018）mg · mL^{-1}，（0.249 ± 0.020）mg · mL^{-1}，（0.233 ± 0.014）mg · mL^{-1}，（0.233 ± 0.011）mg · mL^{-1}，F = 0.511，P = 0.003］。A 组与 B 组比较，差异无统计学意义（P = 0.760）；A 组高于 C 组和 D 组（P = 0.003；P = 0.003）；B 组高于 C 组和 D 组（P = 0.003；P = 0.003）；C 组和 D 组比较，差异无统计学意义（P = 0.751）。③血浆内皮素含量测定结果。4 组大鼠血浆内皮素含量比较，差异有统计学意义［（4.14 ± 0.08）pg · mL^{-1}，（17.75 ± 0.51）pg · mL^{-1}，（19.01 ± 0.13）pg · mL^{-1}，（19.96 ± 0.50）pg · mL^{-1}，F = 4.151，P = 0.001］。A 组低于 B、C、D 组（P = 0.000；P = 0.000；P = 0.000）；B 组低于 C 组和 D 组（P = 0.000；P = 0.000）；C 组低于 D 组（P = 0.000）。④血浆降钙素基因相关肽含量测定结果。4 组大鼠血浆降钙素基因相关肽含量比较，差异有统计学意义［（91.29 ± 0.42）pg · mL^{-1}，（47.99 ± 0.65）pg · mL^{-1}，（56.32 ± 2.17）pg · mL^{-1}，（60.20 ± 0.40）pg · mL^{-1}，F = 2.616，P = 0.003］。A 组高于 B、C、D 组（P = 0.000；P = 0.000；P = 0.000）；B 组低于 C 组和 D 组（P = 0.000；P = 0.000）；C 组低于 D 组（P = 0.000）。⑤血浆内皮素含量和降钙素基因相关肽含量的相关性分析结果。相关性分析结果显示，A、B 组血浆内皮素含量和降钙素基因相关肽含量呈正相关（r = 0.886，P = 0.002；r = 0.989，P = 0.001）；C、D 组血浆内皮素含量和降钙素基因相关肽含量呈负相关（r = −0.706，P = 0.005；r = −0.725，P = 0.007）。⑥舌象及舌组织观察结果。A 组大鼠舌淡红、苔薄白，B 组舌红、苔微黄，C 组舌红、苔腻微黄，D 组舌红、苔黄腻。A 组丝状乳头排列正常，无破坏，乳头呈圆锥形，尖端略向咽部倾斜。B 组丝状乳头复层扁平上皮多有角化、脱落，且乳头高度参差不齐，尖端变钝或消失，上皮的厚度较 C 组变厚。菌状乳头固有层毛细血管增多，乳头数目及其分支减少。C 组丝状乳头复层扁平上皮角化较 B 组严重，上皮形状矮短，尖端变钝或消失。菌状乳头固有层毛细血管增多，乳头数目及其分支增多。D 组丝状乳头复层扁平上皮见角化、脱落，根部间或有空洞表现，上皮形状矮短并多有破坏，尖端变钝间或有消失；菌状乳头固有层毛细血管增多。⑦踝关节滑膜组织观察结果。A 组踝关节及其周围组织结构正常清晰，滑膜完整正常，无任何组织病理学改变；B 组踝关节及其周围组织可见滑膜壁增厚，血管充血明显，滑膜及附近软骨被炎症细胞严重浸润破坏，出现组织坏死现象；C 组表现同 A 组，D 组表现同 B 组；在局部解剖关节取材时，B、D 组踝关节腔内可见尿酸盐结晶沉着。**结论**：在高脂高糖饮食、湿热环境、关节腔注射微晶尿酸钠溶液复合因素干预下，可复制出痛风性关节炎湿热证病证结合大鼠模型。

关键词　关节炎，痛风性　湿热　证候　模型，动物

基金项目：湖南省中医药科研计划项目（2010025），湖南省自然科学基金项目（13JJ3102）

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A rat model of gouty arthritis combined with dampness-heat syndrome *Xiong Hui** , *Qu Liangye* , *Xiang Lili* , *Qi Xinyu* , *Lu Xiaolong* , *Guo Yuxing*. * *The Second Affiliated Hospital of Hunan University of Traditional Chinese Medicine* , *Changsha* 410005 , *Hunan* , *China*

ABSTRACT **Objective**: To explore the method of building a rat model of gouty arthritis combined with dampness-heat syndrome. **Methods**: Forty adult healthy male SD rats (weight 200 +/- 20 g) were randomly divided into 4 groups, 10 rats in each group. Rats in group A and B were fed normally, and rats in group B were administered with intra-articular injection of microcrystalline sodium urate (MSU) solution in the right ankle on the 13th day after the beginning of modeling. Rats in group C and D were fed normally. Meanwhile, they could drink the honey water (200 g/L) freely and they were intragastric administrated with grease (1 g/100 g) and Chinese spirits (52% (vol/vol), 1 mL/100 g) in turn for 10 consecutive days. Since the 11th day, they were fed in the artificial climate box (temperature, 32 +/- 2 degrees centigrade; relative humidity, 92 +/- 3%). Meanwhile, the rats in group D were administered with intra - articular injection of MSU solution in the right ankle on the 13th day. The rats' s diet, mental status, urination and defecation and tongue demonstration were observed during modeling period. The urine contents of aquaporin - 2 (AQP2) were measured on the 15th day from the beginning of modeling. The plasma contents of endothelin (ET) and calcitonin generelated peptide (CGRP) were measured on the 16th day from the beginning of modeling. All rats were executed after the modeling and the pathological changes in tongue tissue and synovial tissue of right ankle joint were observed. **Results**: During feeding period, eating, drinking, activities and excrements of the rats in group A were normal. Eating, drinking, activities and excrements of the rats in group B were normal in the early period after the beginning of the modeling, while since the 13th day, their eating and drinking began to decrease and they became restless. Thickening, red, swelling, hot and action-limited were found in the right ankle joints. After the beginning of modeling, rats in group C presented with reduction in eating and drinking, indulging in lying, sluggish in movement, fluffy and dry hair and yellow urine. On the 5th day from the beginning of modeling, diarrhea or sticky excrements appeared for the rats. On the 11th day, the symptoms of rough breathing, restlessness, loose and rough in hair, flabby and sagging in scrotum and severe diarrhea appeared, and filth were found around the anus. The rats in group D presented with the same symptoms as those of group C after the beginning of modeling, and moreover, presented with the same symptoms as those of group B after the 13th day after modeling. There was statistical difference in the urine content of AQP2 between the 4 groups (0.251 +/- 0.018, 0.249 +/- 0.020, 0.233 +/- 0.014, 0.233 +/- 0.011 mg/mL, $F = 0.511$, $P = 0.003$). There was no statistical difference between group A and group B ($P = 0.760$). Group A surpassed group C and group D ($P = 0.003$; $P = 0.003$). Group B surpassed group C and group D ($P = 0.003$; $P = 0.003$). There was no statistical difference between group C and group D ($P = 0.751$). There was statistical difference in the plasma content of ET between the 4 groups (4.14 +/- 0.08, 17.75 +/- 0.51, 19.01 +/- 0.13, 19.96 +/- 0.50 pg/mL, $F = 4.151$, $P = 0.001$). The plasma content of ET was lower in group A compared with group B, C and D ($P = 0.000$; $P = 0.000$; $P = 0.000$), and was lower in group B compared with group C and group D ($P = 0.000$; $P = 0.000$), and was lower in group C compared with group D ($P = 0.000$). There was statistical difference in the plasma content of CGRP between the 4 groups (91.29 +/- 0.42, 47.99 +/- 0.65, 56.32 +/- 2.17, 60.20 +/- 0.40 pg/mL, $F = 2.616$, $P = 0.003$). The plasma content of CGRP was higher in group A compared with group B, C and D ($P = 0.000$; $P = 0.000$; $P = 0.000$), and was lower in group B compared with group C and group D ($P = 0.000$; $P = 0.000$), and was lower in group C compared with group D ($P = 0.000$). The results of correlation analysis on the plasma content of ET and CGRP showed that they were positively correlated with each other in group A and group B ($r = 0.886$, $P = 0.002$; $r = 0.989$, $P = 0.001$) and were negatively correlated with each other in group C and group D ($r = -0.706$, $P = 0.005$; $r = -0.725$, $P = 0.007$). The results of observation on tongue demonstration and tongue tissue showed that the rats in group A presented with pink tongue and thin white coated tongue, the rats in group B presented with red tongue and yellowish coated tongue, the rats in group C presented with red tongue and yellowish greasy coated tongue, and the rats in group D presented with red tongue and yellow greasy coated tongue. The filiform papillae of the tongue in group A arranged regularly and was not damaged, and the papillas were conical and its tip leaned to the pharynx slightly. The filiform papillae of the tongue in group B presented with stratified squamous epithelium cornification and fallen off, the heights of the papillas were irregular, the tips became blunt or disappeared, and the thickness of epithele became thickening compared with that of group C. The capillaries increased in the lamina propria of the fungiform papillae, and the papillae and its branches decreased. Compared to group B, the keratinization of stratified squamous epithelium of filiform papillae were severer in group C, the epithel was short and its tips were blunt or even disappeared. The capillaries increased in the lamina propria of the fungiform papillae, and the fungiform papillae and its branches increased. The stratified squamous epithelium of filiform papillae of group D were keratinized and fallen off, with cavity in the roots. The epithel was short and was damaged and its tips were blunt or even disap-

peared. The capillaries increased in the lamina propria of the fungiform papillae. The results of observation on synovial tissue of the ankle joint showed that the ankle joints and their surrounding tissues structure were normal and clear in rats of group A, and the synovium were complete and normal without any histopathological changes. The thickened synovial membrane walls and obvious vascular engorgement were found in ankle joints and their surrounding tissues in group B, and the synovial membrane and their vicinal cartilages were infiltrated and damaged by inflammatory cells, and meanwhile, the tissue necrosis were found. The manifestation of group C was the same as that of group A, and the manifestation of group D was the same as that of group B. The urate crystals were found in ankle joint cavities in group B and group D. **Conclusion**: The rat model of gouty arthritis combined with dampness-heat syndrome could be built through intervention of composite factors including high fat and high glucose diet, wet heat environment and intra-articular injection of MSU solution.

Key words　Arthritis, gouty; Dampness - heat; Symptom complex; Models, animal

痛风是嘌呤代谢紊乱和(或)血尿酸升高引起的一组综合征,随着人们生活水平的提高和饮食结构的改变,其发病率逐年上升[1]。目前有关的基础研究和临床研究已成为热点。临床上将痛风性关节炎分为湿热蕴结型、瘀热阻滞型、痰浊阻滞型和肝肾阴虚型,其中湿热蕴结型为临床最常见的痛风性关节炎证候类型[2-3]。但在目前的研究中,基于单纯痛风性关节炎和单纯中医湿热证大鼠模型的研究较多,以痛风性关节炎"病"与中医湿热"证"病证结合动物模型的研究较少。因此,建立痛风性关节炎湿热证病证结合模型已成为亟待解决的问题。本研究拟通过对痛风性关节炎、湿热证单因素造模与多因素复合造模大鼠模型的对比研究,探讨建立痛风性关节炎湿热证病证结合大鼠模型的可行性,现报告如下。

1　材料与仪器

1.1　实验动物　SPF 级健康成年 SD 雄性大鼠 40 只,体质量(200 ± 20) g,由湖南中医药大学动物实验中心提供[SCXH(湘)2009-0004]。

1.2　实验试剂　微晶尿酸钠(microcrystalline sodium urate, MSU)(湖南中医药大学药学院药剂实验室制备),聚山梨酯 80(sigma 公司),52% 红星二锅头酒(北京红星股份有限公司),内皮素(endothelin, ET)放免试剂盒、降钙素基因相关肽(calcitonin generelated peptide, CGRP)放免试剂盒(北京华埠力特生物技术研究所),尿液水通道蛋白 2(aquaporin-2, AQP2)试剂盒(碧云天生物技术研究所),尿酸检测试剂盒(德国罗氏诊断有限公司)。

1.3　实验仪器　QHX-300BS-Ⅲ人工气候箱(上海新苗医疗器械制造有限公司),DNP-9162 型电热恒温箱(上海精宏实验设备有限公司),GC-1500r 放射免疫计数器(科大创新股份有限公司中佳分公司),WH-2 微型漩涡混合仪(上海沪西分析仪器厂有限公司),DNM-9602 酶标分析仪(北京普林新技术有限公司),KDC-2046 低温大容量离心机(中国科学技术力学科技实业总公司中佳光电仪器分公司),美国 A0820 型切片机(美国永生公司),JY3002 型电子天平(上海舜宇恒平科学仪器有限公司),Motic B1 型双目生物显微镜及图像采集和分析软件(麦克奥迪公司)。

2　方　法

2.1　试剂制备　参考 Coderre 等[4]介绍的方法改进制备 MSU 结晶及溶液。将 194 mL 去热源的灭菌注射用水 + 1 mol · L^{-1}的 NaOH 6 mL 煮沸后加入 1 g 尿酸,用 1 mol · L^{-1}HCL 调 pH 值至 7.2,搅拌至冷却,4 ℃冰箱冷藏保存。24 h 后去上清液,用滤纸将沉淀物水分吸干过滤,放入干燥箱 70 ℃烘干 2 h。取出后刮下粉末,放入研器内研成细末,用孔径 200 μm 的金属网过筛,装瓶备用。实验前 MSU 结晶在 180 ℃烘 3 h,90 mg MSU 结晶加 4.5 mL 生理盐水,再加 0.5 mL 聚山梨酯 80,加热搅拌,配成浓度为 20 mg · mL^{-1}的 MSU 溶液。

2.2　动物分组与造模　40 只 SD 大鼠喂养 1 周后采用随机数字表将其分为 4 组,每组 10 只。A 组大鼠在正常温度、湿度(24 ~ 28 ℃,相对湿度 60% ~ 75%)环境下普通饲料喂养、自由饮水 16 d。B 组大鼠在正常温度、湿度环境下以普通饲料喂养、自由饮水 12 d。在第 13 天上午,用带 5 号针头的 1 mL 一次性无菌注射器,在大鼠右侧踝关节背侧,沿 45°方向刺入,至胫骨肌腱内侧,向踝关节腔内注射 20 mg · mL^{-1}的 MSU 溶液 50 μL,制备急性痛风性关节炎模型[5],继续在正常温度、湿度环境下以普通饲料喂养、自由饮水 4 d。C 组和 D 组大鼠在正常温度、湿度环境下,普通饲料喂养,200 g · L^{-1}蜂蜜水自由饮用。每天上午分别以油脂(1 g · 100 g^{-1})和 52% 红星二锅头酒(1 mL · 100 g^{-1})灌胃,二者交替进行,共持续 10 d。自

第 11 天开始每天 8：00—12：00 和 13：00—17：00 将大鼠置于人工气候箱中，温度（32 ±2）℃、相对湿度（92 ±3）%，持续 6 d，期间继续以普通饲料喂养，200 g·L^{-1}蜂蜜水自由饮用。D 组大鼠同时于第 13 天上午按照 B 组的方法制备急性痛风性关节炎模型。

2.3 一般情况观察 以造模开始第 1 天、第 5 天、第 11 天、第 13 天和第 15 天为观察时相点，观察各组大鼠的进食量、饮水量、精神状态及大小便情况。

2.4 尿液 AQP2 含量测定 造模开始后第 15 天，将所有大鼠放入单独的大鼠代谢笼中，禁食，自由饮水，每只收集 24 h 尿液 3.8 ~ 15 mL。将收集到的尿液低温离心 15 min（转速 2 000 r·min^{-1}，离心半径 60 mm），取上清液使尿液浓缩至 1 mL 以下，-20 ℃冰箱保存。采用 Bradford 法测定大鼠尿液中的 AQP2 含量。以 OD 值为横坐标，标准品浓度为纵坐标，建立标准曲线图，并得出标准曲线的直线回归方程，计算样品浓度。

2.5 血浆 ET 含量测定 造模开始后第 16 天，用 10% 水合氯醛（0.36 mL·100 g^{-1}）腹腔注射麻醉，腹主动脉取血 2 mL，注入含 30 μL 7.5% EDTA 二钠和 40 μL 抑肽酶的试管中混匀，在 4 ℃离心 10 min（转速 3 000 r·min^{-1}，离心半径 30 mm），分离血浆。将样品置于 -20 ℃保存，采用放射免疫法测定血浆 ET 含量。

2.6 血浆 CGRP 含量测定 标本采集方法及测定方法同血浆 ET 含量测定。

2.7 舌象及舌组织观察 造模期间肉眼观察大鼠舌象。造模结束后处死大鼠，自舌根部剪下大鼠的舌，以 10% 中性甲醛溶液固定。常规脱钙、石蜡包埋、切片、HE 染色，光学显微镜下观察舌组织病理变化。

2.8 踝关节滑膜组织观察 大鼠处死后，以右侧踝关节为中心分别自其上下 0.5 cm 处剪断，取受试关节和周围软组织，快速切取踝关节滑膜，放入 40 g·L^{-1}中性甲醛溶液中固定，100 g·L^{-1}乙二胺四乙酸脱钙，常规脱水、透明、包埋、切片、HE 染色，光学显微镜下观察滑膜组织病理变化。

2.9 数据统计学处理 采用 SPSS16.0 统计软件对所得数据进行统计分析，4 组大鼠尿液 AQP2、血浆 ET、血浆 CGRP 含量的组间总体比较采用方差分析，组间两两比较采用 LSD-t 检验，血浆 ET 含量和 CGRP 含量的关系的分析采用相关分析法，检验水准 $\alpha = 0.05$。

3 结 果

3.1 一般情况观察结果 A 组大鼠喂养期间，饮食、饮水、活动及大便均正常。B 组大鼠造模前期饮食、饮水、活动及大便均正常，自第 13 天开始饮食、饮水减少，烦躁不安，右踝关节明显增粗，且红、肿、热，伴活动受限。C 组大鼠开始造模后逐渐出现饮食、饮水量减少，嗜卧懒动、行动呆滞，毛发蓬松，颜色枯槁，小便黄；造模第 5 天，开始出现大便溏泻或粘腻；第 11 天出现明显呼吸粗重，烦躁不安，毛发疏松、粗糙，阴囊松弛下垂，大便溏泻或粘腻加重，渐见肛周污秽。D 组大鼠造模后表现与 C 组相同，第 13 天开始合并出现 B 组造模后的相同表现。

3.2 尿液 AQP2 含量测定结果 4 组大鼠尿液 AQP2 含量比较，差异有统计学意义。A 组与 B 组比较，差异无统计学意义（$P = 0.760$）；A 组高于 C 组和 D 组（$P = 0.003$；$P = 0.003$）；B 组高于 C 组和 D 组（$P = 0.003$；$P = 0.003$）；C 组和 D 组比较，差异无统计学意义（$P = 0.751$）。见表 1。

3.3 血浆 ET 含量测定结果 4 组大鼠血浆 ET 含量比较，差异有统计学意义。A 组低于 B、C、D 组（$P = 0.000$；$P = 0.000$；$P = 0.000$）；B 组低于 C 组和 D 组（$P = 0.000$；$P = 0.000$）；C 组低于 D 组（$P = 0.000$）。见表 1。

3.4 血浆 CGRP 含量测定结果 4 组大鼠血浆 CGRP 含量比较，差异有统计学意义。A 组高于 B、C、D 组（$P = 0.000$；$P = 0.000$；$P = 0.000$）；B 组低于 C 组和 D 组（$P = 0.000$；$P = 0.000$）；C 组低于 D 组（$P = 0.000$）。见表 1。

表 1 4 组大鼠尿液 AQP2、血浆 ET、血浆 CGRP 含量

组别	AQP2（mg·mL^{-1}）	ET（pg·mL^{-1}）	CGRP（pg·mL^{-1}）
A 组	0.251 ±0.018	4.14 ±0.08	91.29 ±0.42
B 组	0.249 ±0.020	17.75 ±0.51	47.99 ±0.65
C 组	0.233 ±0.014	19.01 ±0.13	56.32 ±2.17
D 组	0.233 ±0.011	19.96 ±0.50	60.20 ±0.40
F 值	0.511	4.151	2.616
P 值	0.003	0.001	0.003

3.5 血浆 ET 含量和 CGRP 含量的相关性分析结果 相关分析结果显示，A、B 组血浆 ET 含量和 CGRP 含量呈正相关（$r = 0.886$，$P = 0.002$；$r = 0.989$，$P = 0.001$）；C、D 组血浆 ET 含量和 CGRP 含量呈负相关

($r=-0.706$,$P=0.005$;$r=-0.725$,$P=0.007$)。

3.6　舌象及舌组织观察结果　A 组大鼠舌淡红、苔薄白,B 组舌红、苔微黄,C 组舌红、苔腻微黄,D 组舌红、苔黄腻。A 组丝状乳头排列正常,无破坏,乳头呈圆锥形,尖端略向咽部倾斜[图 1(1)、图 2(1)、图 3(1)]。B 组丝状乳头复层扁平上皮多有角化、脱落,且乳头高度参差不齐,尖端变钝或消失,上皮的厚度较 C 组变厚;菌状乳头固有层毛细血管增多,乳头数目及其分支减少[图 1(2)、图 2(2)、图 3(2)]。C 组丝状乳头复层扁平上皮角化较 B 组严重,上皮形状矮短,尖端变钝或消失;菌状乳头固有层毛细血管增多,乳头数目及其分支增多[图 1(3)、图 2(3)、图 3(3)]。D 组丝状乳头复层扁平上皮见角化、脱落,根部间或有空洞表现,上皮形状矮短并多有破坏,尖端变钝间或有消失;菌状乳头固有层毛细血管增多[图 1(4)、图 2(4)、图 3(4)]。

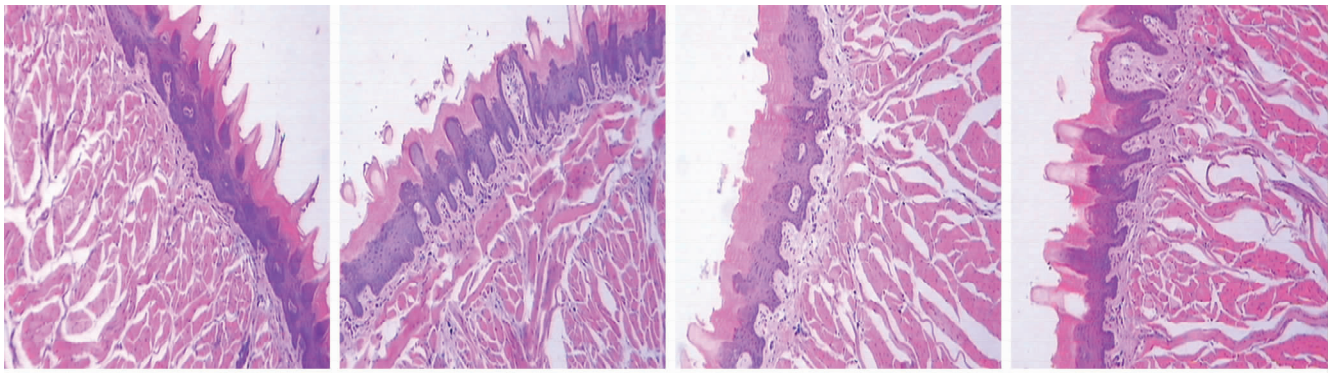


图 1　大鼠舌组织切片(HE 染色　×100)

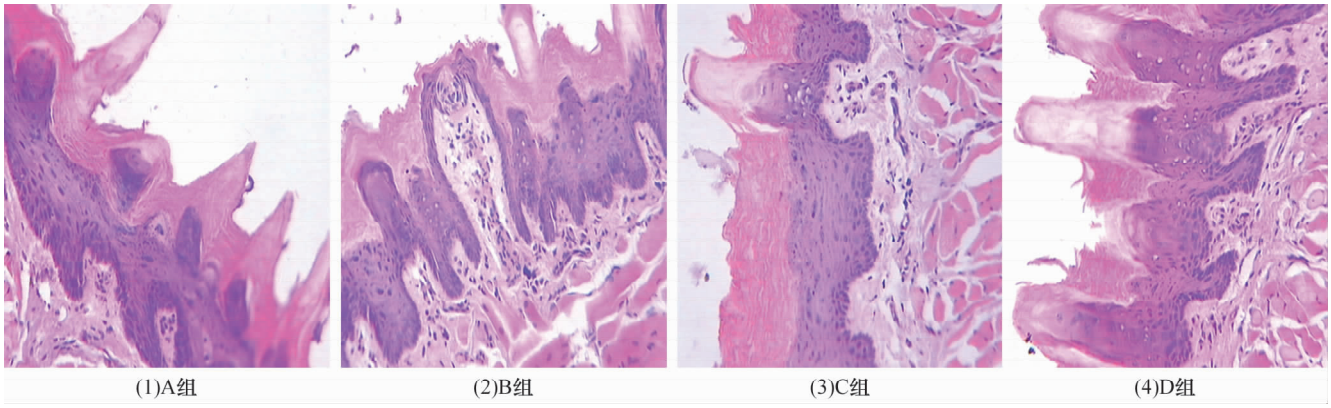


图 2　大鼠舌组织切片(HE 染色　×250)

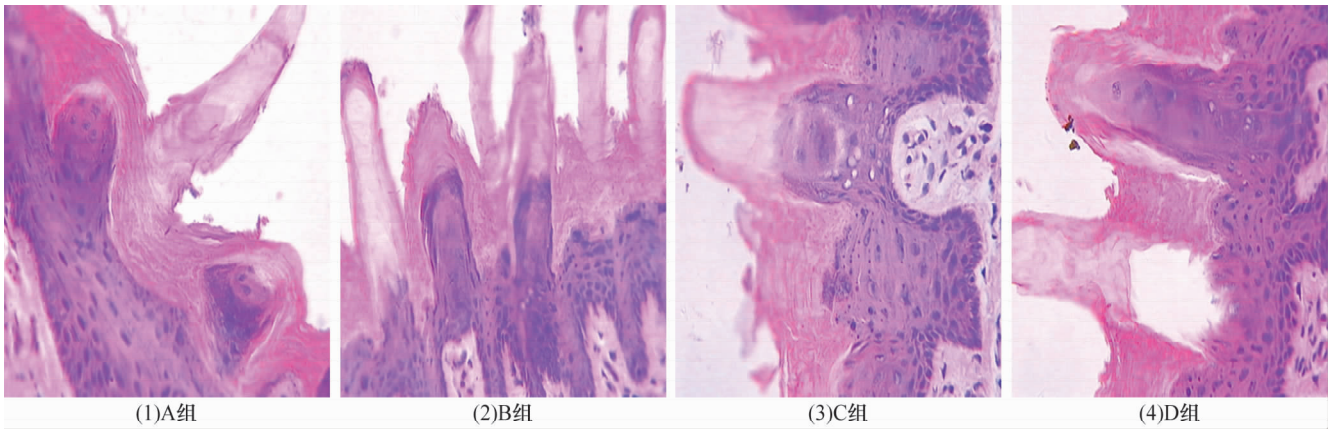
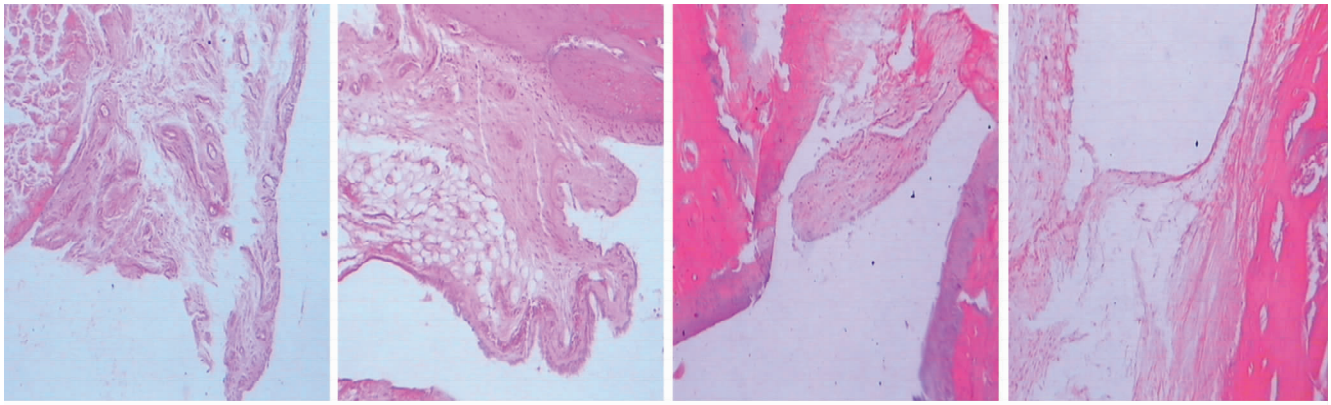


图 3　大鼠舌组织切片(HE 染色　×400)

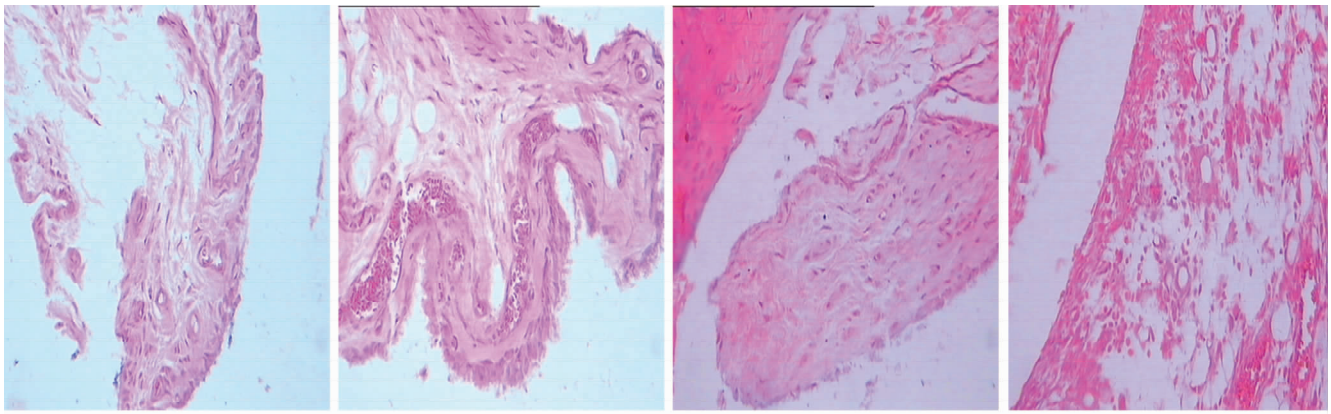
3.7　踝关节滑膜组织观察结果　A 组踝关节及其周围组织结构正常清晰,滑膜完整正常,无任何组织病理学改变[图 4(1)、图 5(1)、图 6(1)]。B 组踝关节及其周围组织可见滑膜壁增厚,血管充血明显,滑膜

及附近软骨被炎症细胞严重浸润破坏，出现组织坏死现象[图 4(2)、图 5(2)、图 6(2)]。C 组表现同 A 组[图 4(3)、图 5(3)、图 6(3)]。D 组表现同 B 组[图 4(4)、图 5(4)、图 6(4)]。在局部解剖关节取材时，B、D 组踝关节腔内可见尿酸盐结晶沉着。



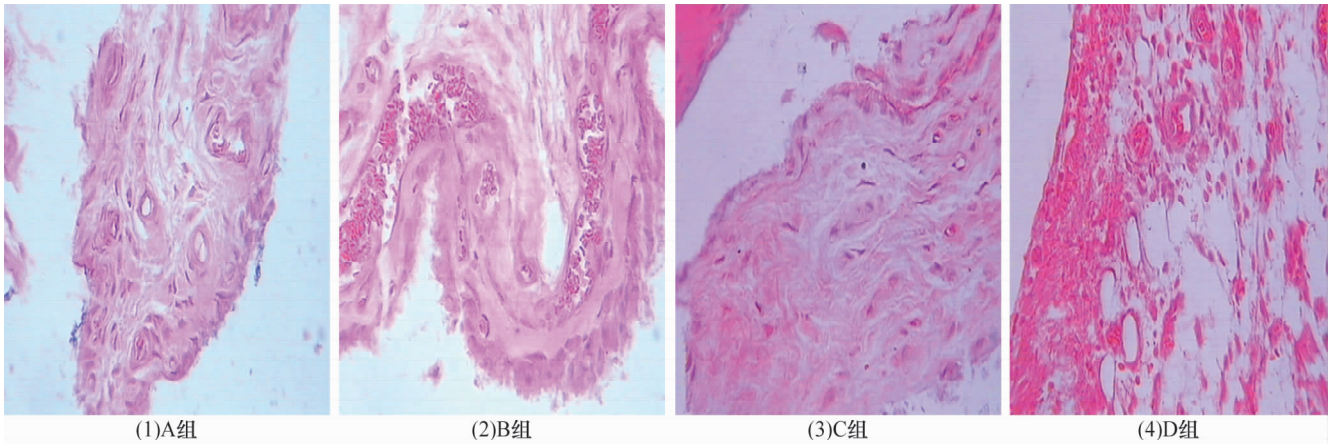
(1)A组　(2)B组　(3)C组　(4)D组

图 4　大鼠踝关节滑膜组织切片(HE 染色 ×100)



(1)A组　(2)B组　(3)C组　(4)D组

图 5　大鼠踝关节滑膜组织切片(HE 染色 ×250)



(1)A组　(2)B组　(3)C组　(4)D组

图 6　大鼠踝关节滑膜组织切片(HE 染色 ×400)

4　讨　论

中医古籍中记载的“历节”“白虎历节”“白虎风”等病与痛风性关节炎相似，历代医家如张仲景、朱丹溪等都在自己的著作中对其进行了论述。湿热蕴结型痛风性关节炎是临床最常见的痛风性关节炎证候类型，建立与其相对应的痛风性关节炎湿热证病证结合动物模型具有重要的临床和科研价值。

察舌辨证是中医学临床诊疗的重要内容，中医湿热证舌象为舌质红、苔黄腻。但是大鼠舌象凭借肉眼难以准确鉴定，所以本研究将舌的组织形态学改变作

为研究湿热证的特异性指标。舌面有许多乳头状隆起,称为舌乳头,包括丝状乳头、菌状乳头和轮廓乳头。其中,丝状乳头和菌状乳头与舌象的形成有密切的联系。丝状乳头呈圆锥形,尖端略向咽部倾斜,它的复层扁平上皮常有角化和脱落,再混以食物残渣、唾液等,使舌黏膜表面覆以一层白色薄苔,称为舌苔。菌状乳头散在于丝状乳头之间,呈蘑菇状,上皮表面比较平滑,固有层富含毛细血管,使乳头外观呈红色,肉眼观察呈红色小点,它的形态及色泽改变是引起舌质变化的主要因素。腻苔是由于乳头数目及其分支增加所致,舌质的颜色则多由舌黏膜固有层及肌层血运情况决定。舌象肉眼观察结果显示,A 组大鼠对应的舌象为舌淡红、苔薄白,B 组为舌红、苔微黄,C 组为舌红、苔腻微黄,D 组为舌红、苔黄腻。光学显微镜下的舌组织观察结果与通过肉眼观察的舌象基本一致,提示在高脂高糖饮食、湿热环境综合影响下大鼠表现出了湿热证舌象,即舌质红、苔黄腻。而 D 组大鼠的一般状态也与痛风性关节炎湿热证的表现相符[6]。

AQP2 是近年来发现的存在于肾脏集合管的调节水重吸收的关键蛋白之一[7]。吴仕九等[8]的研究表明,尿液 AQP2 含量可作为判断湿热证“湿”邪偏重与否的重要指标。C、D 组尿液 AQP2 含量低于 A 组和 B 组,且 C、D 组比较,差异无统计学意义。提示此实验成功复制出痛风性关节炎湿热证中的“湿”证。

ET 具有强烈、持久的缩血管作用,而 CGRP 则是目前已知的最强的舒血管物质之一,对 ET 具有生物学拮抗作用[9]。ET 和 CGRP 可通过调节全身血管收缩与舒张,影响血流量及机体散热[10-13],ET 含量升高、CGRP 含量下降可使全身血管收缩明显大于舒张,散热受抑制,与湿热证“热”的机制相关[14]。本研究中 C、D 组大鼠血浆 ET 含量高于 A 组,CGRP 含量低于 A 组,且 C、D 组大鼠血浆 ET 含量和 CGRP 含量呈负相关,ET 升高、CGRP 下降使得全身血管收缩明显大于舒张,散热受抑制,提示该实验成功复制出痛风性关节炎湿热证中的“热” 证;而 C、D 组大鼠血浆 ET 含量和 CGRP 含量呈负相关,与吴仕九等[14]的研究结果一致。

在局部解剖关节取材时,B、D 组大鼠踝关节腔内可见尿酸盐结晶沉着。光镜下显示关节滑膜壁增厚,血管充血明显,滑膜及附近软骨被炎症细胞严重浸润破坏,出现组织坏死现象,符合尿酸钠结晶诱导大鼠急性痛风性关节炎模型的滑膜病理表现[5]。

本研究结果提示,在高脂高糖饮食、湿热环境、关节腔注射 MSU 溶液复合因素干预下,可复制出痛风性关节炎湿热证病证结合大鼠模型。

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(2013-08-07 收稿　2014-02-12 修回)