·基础研究·

蠲痹历节清方干预鸡急性痛风性关节炎模型的实验研究

齐新宇[1],熊辉[2],周彪[1],向黎黎[1],李腾龙[1],郭玉星[1],陆小龙[2]

（1.湖南中医药大学,湖南　长沙　410208;
2.湖南中医药大学第二附属医院,湖南　长沙　410005）

摘　要　目的:观察蠲痹历节清方干预鸡急性痛风性关节炎模型的疗效,并探讨其可能的作用机制。**方法:**将 160 只 30 日龄雄性湘黄鸡随机分为对照组、模型组、蠲痹历节清方组及别嘌醇组,每组 40 只。对照组以正常饲料喂养;其余 3 组以蛋白含量为 50% 的饲料喂养,连续喂养 21 d 制成急性痛风性关节炎模型。自造模结束后第 1 天开始,蠲痹历节清方组及别嘌醇组分别以蠲痹历节清方汤剂和别嘌醇溶液灌胃,其余 2 组均以生理盐水灌胃,每日 2 次,共 21 d。分别于药物干预开始后 1、7、14、21 d 在每组随机选出 10 只鸡,分别观察其一般状态、踝关节周径、血尿酸含量和血清黄嘌呤氧化酶(xanthine oxidase,XOD)活性,随后处死动物,分离出左侧踝关节滑膜组织,光镜下观察其滑膜组织形态。**结果:**①一般状态。除对照组外,其余 3 组动物药物干预开始时精神萎顿,羽毛松乱、灰暗无光泽,饮食减退,双膝与双踝关节肿大,跛行,站立不稳,粪便中白色物质增多。至药物干预后 21 d 时,蠲痹历节清方组和别嘌醇组动物一般状态基本恢复正常;模型组动物一般状态较开始时好转,但表现较蠲痹历节清方组和别嘌醇组差;对照组动物除体质量增加外,其余表现与造模前一致。造模及实验过程中各组均无动物死亡。②踝关节周径。药物干预开始后各时点,4 组动物踝关节周径比较,组间差异均有统计学意义($F=25.172$,$P=0.000$;$F=24.445$,$P=0.000$;$F=21.237$,$P=0.014$;$F=29.881$,$P=0.041$)。药物干预开始后 1、7、14 d 时,模型组、蠲痹历节清方组及别嘌醇组踝关节周径均大于对照组($P=0.001$,$P=0.001$,$P=0.001$;$P=0.002$,$P=0.002$,$P=0.001$;$P=0.001$,$P=0.002$,$P=0.001$);药物干预开始后 7、14 d 时,蠲痹历节清方组和别嘌醇组踝关节周径均小于模型组($P=0.025$,$P=0.014$;$P=0.012$,$P=0.011$);药物干预开始后 21 d 时,对照组、蠲痹历节清方组及别嘌醇组踝关节周径均小于模型组($P=0.001$,$P=0.015$,$P=0.013$);其余各时点各组间两两比较,组间差异均无统计学意义。③血尿酸含量。药物干预开始后各时点,4 组动物血尿酸含量比较,组间差异均有统计学意义($F=25.361$,$P=0.003$;$F=32.371$,$P=0.005$;$F=36.734$,$P=0.021$;$F=48.336$,$P=0.023$)。药物干预开始后 1、7、14 d 时,模型组、蠲痹历节清方组及别嘌醇组血尿酸含量均大于对照组($P=0.000$,$P=0.001$,$P=0.001$;$P=0.001$,$P=0.001$,$P=0.002$;$P=0.001$,$P=0.000$,$P=0.001$);药物干预开始后 7、14 d 时,蠲痹历节清方组和别嘌醇组血尿酸含量均小于模型组($P=0.011$,$P=0.010$;$P=0.014$,$P=0.002$);药物干预开始后 21 d 时,对照组、蠲痹历节清方组及别嘌醇组血尿酸含量均小于模型组($P=0.001$,$P=0.012$,$P=0.011$);其余各时点各组间两两比较,组间差异均无统计学意义。④血清 XOD 活性。药物干预开始后各时点,4 组动物血清 XOD 活性比较,组间差异均有统计学意义($F=45.721$,$P=0.001$;$F=50.634$,$P=0.002$;$F=49.448$,$P=0.013$;$F=63.124$,$P=0.027$)。药物干预开始后 1、7、14 d 时,模型组、蠲痹历节清方组及别嘌醇组血清 XOD 活性均高于对照组($P=0.002$,$P=0.001$,$P=0.001$;$P=0.001$,$P=0.000$,$P=0.001$;$P=0.001$,$P=0.001$,$P=0.000$);药物干预开始后 7、14 d 时,蠲痹历节清方组和别嘌醇组血清 XOD 活性均低于模型组($P=0.001$,$P=0.013$;$P=0.002$,$P=0.015$);药物干预开始后 21 d 时,对照组、蠲痹历节清方组及别嘌醇组血清 XOD 活性均低于模型组($P=0.001$,$P=0.013$,$P=0.017$);其余各时点各组间两两比较,组间差异均无统计学意义。⑤滑膜组织形态。药物干预开始后各时点,4 组动物滑膜中血管数量比较,组间差异均有统计学意义($F=24.772$,$P=0.032$;$F=33.176$,$P=0.021$;$F=32.672$,$P=0.003$;$F=44.351$,$P=0.000$)。药物干预开始后 1、7、14 、21 d 时,模型组、蠲痹历节清方组及别嘌醇组滑膜中血管数量均多于对照组($P=0.002$,$P=0.001$,$P=0.001$;$P=0.001$,$P=0.001$,$P=0.002$;$P=0.001$,$P=0.000$,$P=0.001$;$P=0.001$,$P=0.003$,$P=0.002$);药物干预开始后 7、14、21 d 时,蠲痹历节清方组和别嘌醇组滑膜中血管数量均多于模型组($P=0.001$,$P=0.013$;$P=0.014$,$P=0.011$;$P=0.001$,$P=0.012$);其余各时点各组间两两比较,组间差异均无统计学意义。药物干预开始后各时点,4 组动物滑膜中中性粒细胞数量比较,组间差异均有统计学意义($F=32.347$,$P=0.001$;$F=43.561$,$P=0.001$;$F=42.361$,$P=0.014$;$F=51.745$,$P=0.011$)。药物干预开始后 1、7、14 d 时,模型组、蠲痹历节清方组及别嘌醇组滑膜中中性粒细胞数量均多于对照组($P=0.001$,$P=0.002$,$P=0.001$;$P=0.001$,$P=0.001$,$P=0.002$;$P=0.002$,$P=0.001$,$P=$

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通讯作者:熊辉　E-mail:xh_hn@sina.com

0.001);药物干预开始后 7、14 d 时,蠲痹历节清方组和别嘌醇组滑膜中中性粒细胞数量均少于模型组($P=0.017$,$P=0.014$;$P=0.012$,$P=0.014$);药物干预开始后 21 d 时,对照组、蠲痹历节清方组及别嘌醇组滑膜中中性粒细胞数量均少于模型组($P=0.001$,$P=0.013$,$P=0.017$);其余各时点各组间两两比较,组间差异均无统计学意义。**结论**:蠲痹历节清方可通过抑制 XOD 活性,有效降低血尿酸水平,缓解鸡急性痛风性关节炎模型临床症状,其疗效与别嘌醇相当。

关键词　关节炎,痛风性;蠲痹历节清方;疾病模型,动物;尿酸;黄嘌呤氧化酶;动物实验

Juanbilijieqing Fang(蠲痹历节清方) interfere with acute gouty arthritis chiken model

QI Xinyu[1],XIONG Hui[2],ZHOU Biao[1],XIANG Lili[1],LI Tenglong[1],GUO Yuxing[1],LU Xiaolong[2]

1. Hunan University of Traditional Chinese Medicine,Changsha 410208,Hunan,China

2. The Second Affiliated Hospital of Hunan University of Traditional Chinese Medicine,Changsha 410005,Hunan,China

ABSTRACT　**Objective**:Objective: To observe the effect of Juanbilijieqing Fang(蠲痹历节清方) on acute gouty arthritis chiken model and to explore the mechanism of action. **Methods**:One hundred and sixty 30 - day - old male XIANGHUANG chickens were randomly divided into control group,model group,Juanbilijieqing group and allopurinol group,40 cases in each group. The chickens in control group were fed with normal feedstuff,while the chickens in other groups were fed for consecutive 21 days with feedstuff which protein content was 50% to made into acute gouty arthritis models. After the end of the modeling,the chickens in Juanbilijieqing group and allopurinol group were intragastric administrated with Juanbilijieqing decoction and allopurinol solution respectively,while chickens in the other 2 groups were intragastric administrated with normal saline,twice a day for 21 consecutive days. Ten chickens were randomly selected from each group at 1,7,14 and 21 days after the beginning of drug intervention respectively;and the general state,ankle circumference,blood uric acid levels and serum xanthine oxidase(XOD) activity were observed respectively. Then the chickens were executed and the left ankle synovium were separated for observing the synovial tissue morphology under the optical microscope. **Results**:The chickens except those in control group became lethargic and their feathers became disorderly loose and matte and their eating and drinking began to decrease. Both knees and ankles began to swell up,and limping and unstable standing were found. White matters increased in feces. The general state of chickens in Juanbilijieqing group and allopurinol group basically returned to normal at 21 days after the beginning of the drug intervention. The chickens in model group improved in the general state,while their performance were worse than that of chickens in Juanbilijieqing group and allopurinol group. No change except increased body mass were found in the chickens in control group. No chickens died in each group during the modeling and experimental process. There was statistical difference in ankle circumference between the 4 groups at different timepoints after the beginning of the drug intervention($F=25.172$,$P=0.000$;$F=24.445$,$P=0.000$;$F=21.237$,$P=0.014$;$F=29.881$,$P=0.041$). The ankle circumference was greater in model group,Juanbilijieqing group and allopurinol group compared with control group at 1,7 and 14 days after the beginning of the drug intervention($P=0.001$,$P=0.001$,$P=0.001$;$P=0.002$,$P=0.002$,$P=0.001$;$P=0.001$,$P=0.002$,$P=0.001$). The ankle circumference was less in Juanbilijieqing group and allopurinol group compared with model group at 7 and 14 days after the beginning of the drug intervention($P=0.025$,$P=0.014$;$P=0.012$,$P=0.011$). The ankle circumference was less in control group,Juanbilijieqing group and allopurinol group compared with model group at 21 days after the beginning of the drug intervention($P=0.001$,$P=0.015$,$P=0.013$). There was no statistical difference in the ankle circumference between the paired groups at other timepoints. There was statistical difference in blood uric acid levels between the 4 groups at different timepoints after the beginning of the drug intervention($F=25.361$,$P=0.003$;$F=32.371$,$P=0.005$;$F=36.734$,$P=0.021$;$F=48.336$,$P=0.023$). The blood uric acid levels were greater in model group,Juanbilijieqing group and allopurinol group compared with control group at 1,7 and 14 days after the beginning of the drug intervention($P=0.000$,$P=0.001$,$P=0.001$;$P=0.001$,$P=0.001$,$P=0.002$;$P=0.001$,$P=0.000$,$P=0.001$). The blood uric acid levels were less in Juanbilijieqing group and allopurinol group compared with model group at 7 and 14 days after the beginning of the drug intervention($P=0.011$,$P=0.010$;$P=0.014$,$P=0.002$). The blood uric acid levels were less in control group,Juanbilijieqing group and allopurinol group compared with model group at 21 days after the beginning of the drug intervention($P=0.001$,$P=0.012$,$P=0.011$). There was no statistical difference in the blood uric acid levels between the paired groups at other timepoints. There was statistical difference in serum XOD activity between the 4 groups at different timepoints after the beginning of the drug intervention($F=45.721$,$P=0.001$;$F=50.634$,$P=0.002$;$F=49.448$,$P=0.013$;$F=63.124$,$P=0.027$). The serum XOD activity was greater in model group,Juanbilijieqing group and allopurinol group compared with control group at 1,7 and 14 days after the beginning of the drug intervention($P=0.002$,$P=0.001$,$P=0.001$;$P=0.001$,$P=0.000$,$P=0.001$;$P=0.001$,$P=0.001$,$P=0.000$). The serum XOD activity was less

in Juanbilijieqing group and allopurinol group compared with model group at 7 and 14 days after the beginning of the drug intervention(P = 0.001,P = 0.013;P = 0.002,P = 0.015). The serum XOD activity was less in control group,Juanbilijieqing group and allopurinol group compared with model group at 21 days after the beginning of the drug intervention(P = 0.001,P = 0.013,P = 0.017). There was no statistical difference in serum XOD activity between the paired groups at other timepoints. There was statistical difference in the number of blood vessels in synovium between the 4 groups at different timepoints after the beginning of the drug intervention(F = 24.772,P = 0.032;F = 33.176,P = 0.021;F = 32.672,P = 0.003;F = 44.351,P = 0.000). The number of blood vessels in synovium was more in model group,Juanbilijieqing group and allopurinol group compared with control group at 1,7,14 and 21 days after the beginning of the drug intervention (P = 0.002,P = 0.001,P = 0.001;P = 0.001,P = 0.001,P = 0.002;P = 0.001,P = 0.000,P = 0.001;P = 0.001,P = 0.003,P = 0.002). The number of blood vessels in synovium was more in Juanbilijieqing group and allopurinol group compared with model group at 7,14 and 21 days after the beginning of the drug intervention(P = 0.001,P = 0.013;P = 0.014,P = 0.011;P = 0.001,P = 0.012). There was no statistical difference in the number of blood vessels in synovium between the paired groups at other timepoints. There was statistical difference in the number of neutrophils in synovium between the 4 groups at different timepoints after the beginning of the drug intervention (F = 32.347,P = 0.001;F = 43.561,P = 0.001;F = 42.361,P = 0.014;F = 51.745,P = 0.011). The number of neutrophils in synovium was more in model group,Juanbilijieqing group and allopurinol group compared with control group at 1,7 and 14 days after the beginning of the drug intervention(P = 0.001,P = 0.002,P = 0.001;P = 0.001,P = 0.001,P = 0.002;P = 0.002,P = 0.001,P = 0.001). The number of neutrophils in synovium was less in Juanbilijieqing group and allopurinol group compared with model group at 7 and 14 days after the beginning of the drug intervention(P = 0.017,P = 0.014;P = 0.012,P = 0.014). The number of neutrophils in synovium was less in control group,Juanbilijieqing group and allopurinol group compared with model group at 21 days after the beginning of the drug intervention(P = 0.001,P = 0.013,P = 0.017). There was no statistical difference in the number of neutrophils in synovium between the paired groups at other timepoints. **Conclusion**:Juanbilijieqing Fang can effectively reduce the blood uric acid levels and relieve the clinical symptoms of acute gouty arthritis models through decreasing the activity of XOD,and it is similar to allopurinol in the curative effect.

Key words arthritis,gouty;Juanbilijieqing Fang;disease models,animal;uric acid;xanthine oxidase;animal experimentation

痛风性关节炎是由于尿酸钠盐在细胞外液中过度饱和沉积于关节而引起的一种非特异性晶体相关性关节病,其发生与体内嘌呤代谢障碍和(或)尿酸排泄减少致血尿酸升高有关[1],近年来国内该病的发病率逐年升高[2]。我们根据痛风性关节炎的病机及发病特点,在临床应用中药蠲痹历节清方治疗急性痛风性关节炎取得了满意的临床疗效[3]。为验证其疗效及探讨可能的作用机制,我们进行了蠲痹利节清方干预鸡急性痛风性关节炎模型动物实验,现将其报告如下。

1 材料与仪器

1.1 实验动物 30 日龄 SPF 级健康雄性湘黄鸡 160 只,体质量(500 ± 50)g,由湖南中医药大学动物实验中心代购,合格证号:2014 - 0004。实验通过医学实验动物伦理委员会批准。

1.2 实验药物及试剂 蠲痹历节清方,方药组成:苍术 20 g、黄芩 10 g、黄柏 10 g、防己 10 g、土茯苓 15 g、茵陈 15 g、泽泻 10 g、白术 10 g、当归 15 g、甘草 6 g,生药购自湖南中医药大学第二附属医院,由湖南中医药大学药学院统一煎制、浓缩至含生药 0.83 g·mL^{-1},4 ℃保存;别嘌醇片(广东彼迪药业有限公司,批号 H44021368),以蒸馏水配置成 1.95 mg·mL^{-1},4 ℃保存;水合氯醛(天津科密欧化学试剂有限公司,批号 20120303);苏木素(武汉德士生物工程有限公司,批号 20121227);甲醛(湖南医科大学防疫制品厂,批号 98091002);尿酸测定试剂盒(南京建成生物工程研究所,批号 K608 - 100);黄嘌呤氧化酶(Xanthine oxidase,XOD)测试盒(南京建成生物工程研究所,批号 20131022)。

1.3 实验仪器 HHS - 2 电子恒温不锈钢水浴锅(上海南阳仪器有限公司);JY3002 型电子天平(上海精密科学仪器有限公司);LEICA DM LB2 型双目显微镜(LEICA 公司);MIAS 医学图象分析系统(北航公司);Haier 医用微波炉(Haier 集团);DT5 - 3 台式离心机(北京时代北利离心机有限公司);S2 - 93 自动双重纯水蒸馏器(上海亚荣生化仪器厂);Shandon325 型石蜡切片机(Shandon 公司);DNP - 9162 型电热恒温培养箱(上海精宏实验设备有限公司);Motic B5 显微摄像系统(麦克奥迪实业集团公司)。

2 方 法

2.1 饲料配制 正常饲料(湖南省农科院畜牧所饲

料厂提供,批号:20140803),蛋白含量 19.8%、钙含量 5%;模型饲料参考文献[4-7]进行配置,以 15 kg 为 1 个基本单位,含鱼粉 12.50 kg、石粉 2.50 kg,蛋白含量 50%、钙含量 9.17%。

2.2　动物分组与造模　所有动物在相同环境中以正常饲料适应性喂养,1 周后采用随机数字表将 160 只湘黄鸡随机分为对照组、模型组、蠲痹历节清方组及别嘌醇组,每组 40 只。对照组继续以正常饲料喂养,其余 3 组按照文献[6-7]制备急性痛风性关节炎模型的方法,以模型饲料喂养。所有动物均保证每日食量,每天饮水量≤100 mL,连续喂养 21 d。

2.3　药物干预　自造模结束后第 1 天开始,蠲痹历节清方组及别嘌醇组分别以蠲痹历节清方汤剂和别嘌醇溶液灌胃,根据人与动物之间药物等效剂量换算公式[8]计算结果,分别给予蠲痹历节清方汤剂(30 mL·kg^{-1})和别嘌醇(15 mL·kg^{-1})灌胃,其余 2 组均以等体积生理盐水灌胃,每日 2 次,共 21 d。

2.4　实验观察　分别于药物干预开始后 1、7、14、21 d在每组随机选出 10 只鸡,分别观察其一般状态、踝关节周径、血尿酸含量和血清 XOD 活性,随后处死动物,分离出左侧踝关节滑膜组织,光镜下观察其滑膜组织形态。一般状态观察包括其精神状态、外形改变、活动状态、饮食与及排便情况[9-10]。踝关节周径采用软皮尺测量,以左侧踝关节紧靠踝部第 1 足爪下方环绕跗踝部的周长为踝关节周径,测 2 次取平均值。测定血尿酸含量及血清 XOD 活性时,分别在其腋下抽取腋下静脉血约 4 mL,冷藏后统一采用酶学比色法检测。滑膜组织形态观察主要包括光学显微镜下滑膜病理改变观察与滑膜中血管和中性粒细胞数量的统计。

2.5　数据统计分析　采用 SPSS17.0 软件进行统计分析,4 组动物踝关节周径、血尿酸含量、血清 XOD 活性、踝关节滑膜中血管数量和中性粒细胞数量的组间比较采用单因素方差分析,组间两两比较采用 LSD-t 检验,检验水准 $\alpha=0.05$。

3　结　果

3.1　一般状态　药物干预开始后 7 d 时,蠲痹历节清方组和别嘌醇组动物饮食量增加,大便成形,粪便中白色物质减少;药物干预开始后 14 d,饮食量明显增加,粪便中白色物质明显减少,素囊饱满,活动量增加,精神状态好转,关节肿胀情况好转;药物干预开始后 21 d,蠲痹历节清方组和别嘌醇组动物羽毛光泽恢复,食量明显增加、素囊饱满,鸡冠转红,双膝与双踝关节肿大消退,活动量增加,体质量增加,开始打鸣,粪便中白色物质消失;模型组动物一般状态较造模结束时好转,但表现较蠲痹历节清方组和别嘌醇组差;对照组动物除体质量增加外,其余表现与造模前一致。造模及实验过程中各组均无动物死亡。

3.2　踝关节周径　药物干预开始后各时点,4 组动物踝关节周径比较,差异均有统计学意义。药物干预开始后 1、7、14 d 时,模型组、蠲痹历节清方组及别嘌醇组踝关节周径均大于对照组($P=0.001$,$P=0.001$,$P=0.001$;$P=0.002$,$P=0.002$,$P=0.001$;$P=0.001$,$P=0.002$,$P=0.001$);药物干预开始后 7、14 d 时,蠲痹历节清方组和别嘌醇组踝关节周径均小于模型组($P=0.025$,$P=0.014$;$P=0.012$,$P=0.011$);药物干预开始后 21 d 时,对照组、蠲痹历节清方组及别嘌醇组踝关节周径均小于模型组($P=0.001$,$P=0.015$,$P=0.013$);其余各时点各组间两两比较,差异均无统计学意义。见表 1。

3.3　血尿酸含量　药物干预开始后各时点,4 组动物血尿酸含量比较,差异均有统计学意义。药物干预开始后 1、7、14 d 时,模型组、蠲痹历节清方组及别嘌醇组血尿酸含量均大于对照组($P=0.000$,$P=0.001$,$P=0.001$;$P=0.001$,$P=0.001$,$P=0.002$;$P=0.001$,$P=0.000$,$P=0.001$);药物干预开始后 7、

表 1　4 组动物药物干预开始后不同时点踝关节周径的比较　$\bar{x}\pm s$,cm

组别	样本量(只)	药物干预开始后时间点			
		1 d	7 d	14 d	21 d
对照组	10	7.05±0.82	7.21±0.43	7.35±0.28	7.50±0.21
模型组	10	10.57±0.33	9.94±0.18	9.43±0.43	8.86±0.31
蠲痹历节清方组	10	10.63±0.61	9.43±0.74	8.21±0.39	7.71±0.17
别嘌醇组	10	10.61±0.52	9.46±0.67	8.24±0.79	7.79±0.24
F 值		25.172	24.445	21.237	29.881
P 值		0.000	0.000	0.014	0.041

14 d 时，蠲痹历节清方组和别嘌醇组血尿酸含量均小于模型组（$P = 0.011$，$P = 0.010$；$P = 0.014$，$P = 0.002$）；药物干预开始后 21 d 时，对照组、蠲痹历节清方组及别嘌醇组血尿酸含量均小于模型组（$P = 0.001$，$P = 0.012$，$P = 0.011$）；其余各时点各组间两两比较，差异均无统计学意义。见表 2。

表 2　4 组动物药物干预开始后不同时点血尿酸含量的比较　$\bar{x} \pm s$，μmol · L^{-1}

组别	样本量（只）	药物干预开始后时间点			
		1 d	7 d	14 d	21 d
对照组	10	194.70 ± 0.77	189.37 ± 0.65	188.70 ± 1.27	189.64 ± 3.31
模型组	10	511.89 ± 0.87	489.52 ± 5.20	441.49 ± 4.76	392.43 ± 5.51
蠲痹历节清方组	10	512.78 ± 0.45	423.53 ± 5.67	353.78 ± 5.65	240.74 ± 4.61
别嘌醇组	10	509.65 ± 0.29	434.56 ± 5.21	362.35 ± 4.74	252.65 ± 5.62
F 值		25.361	32.371	36.734	48.336
P 值		0.003	0.005	0.021	0.023

3.4　血清 XOD 活性　药物干预开始后各时点，4 组动物血清 XOD 活性比较，差异均有统计学意义。药物干预开始后 1、7、14 d 时，模型组、蠲痹历节清方组及别嘌醇组血清 XOD 活性均高于对照组（$P = 0.002$，$P = 0.001$，$P = 0.001$；$P = 0.001$，$P = 0.000$，$P = 0.001$；$P = 0.001$，$P = 0.001$，$P = 0.000$）；药物干预开始后 7、14 d 时，蠲痹历节清方组和别嘌醇组血清 XOD 活性均低于模型组（$P = 0.001$，$P = 0.013$；$P = 0.002$，$P = 0.015$）；药物干预开始后 21 d 时，对照组、蠲痹历节清方组及别嘌醇组血清 XOD 活性均低于模型组（$P = 0.001$，$P = 0.013$，$P = 0.017$）；其余各时点各组间两两比较，差异均无统计学意义。见表 3。

表 3　4 组动物药物干预开始后不同时点血清 XOD 活性的比较　$\bar{x} \pm s$，U · L^{-1}

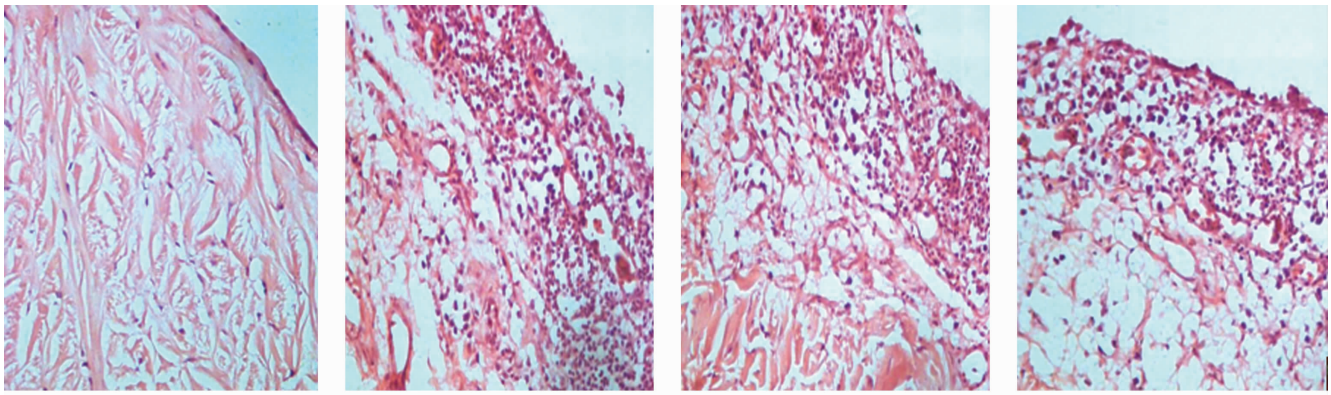
组别	样本量（只）	药物干预开始后时间点			
		1 d	7 d	14 d	21 d
对照组	10	25.01 ± 2.10	25.91 ± 2.41	25.93 ± 3.82	26.35 ± 3.62
模型组	10	55.07 ± 3.46	51.96 ± 3.82	46.89 ± 4.32	41.07 ± 4.23
蠲痹历节清方组	10	56.54 ± 3.53	45.11 ± 5.52	36.26 ± 3.46	29.14 ± 3.12
别嘌醇组	10	54.67 ± 4.62	45.63 ± 4.94	37.32 ± 3.27	31.06 ± 3.45
F 值		45.721	50.634	49.448	63.124
P 值		0.001	0.002	0.013	0.027

3.5　滑膜组织形态　药物干预开始后 1 d 时，对照组踝关节滑膜层内膜由 1 ~ 3 层细胞组成，结构完整、层次分明；其余 3 组踝关节滑膜组织均出现组织结构破坏严重，层次紊乱，增厚，滑膜下层细胞数量显著增多，部分呈束状改变，可见大量毛细血管增生并充血扩张，同时可见大量中性粒细胞浸润（图 1）。药物干预开始后 21 d 时，对照组踝关节滑膜组织与药物干预开始后 1 d 时相比，无明显变化；模型组滑膜组织结构破坏，稍增厚，滑膜层次紊乱，滑膜下层细胞数量增多，部分呈束状改变，可见少量毛细血管增生并小血管充血扩张，同时可见大量中性粒细胞浸润；其余 2 组滑膜组织结构完整、增厚，可见大量血管增生并小血管稍充血扩张，同时可见少量中性粒细胞浸润（图 2）。

药物干预开始后各时点，4 组动物滑膜中血管数量比较，差异均有统计学意义。药物干预开始后 1、7、14 、21 d 时，模型组、蠲痹历节清方组及别嘌醇组滑膜中血管数量均多于对照组（$P = 0.002$，$P = 0.001$，$P = 0.001$；$P = 0.001$，$P = 0.001$，$P = 0.002$；$P = 0.001$，$P = 0.000$，$P = 0.001$；$P = 0.001$，$P = 0.003$，$P = 0.002$）；药物干预开始后 7、14、21 d 时，蠲痹历节清方组和别嘌醇组滑膜中血管数量均多于模型组（$P = 0.001$，$P = 0.013$；$P = 0.014$，$P = 0.011$；$P = 0.001$，$P = 0.012$）；其余各时点各组间两两比较，差异均无统计学意义。见表 4。

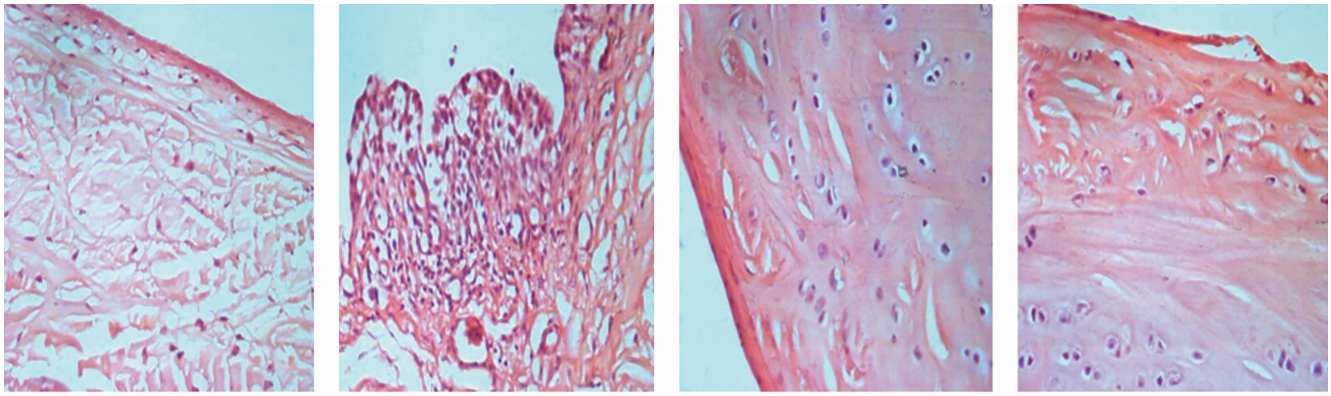
药物干预开始后各时点，4 组动物滑膜中中性粒细胞数量比较，差异均有统计学意义。药物干预开始后 1、7、14 d 时，模型组、蠲痹历节清方组及别嘌醇组滑膜中中性粒细胞数量均多于对照组（$P = 0.001$，$P = 0.002$，$P = 0.001$；$P = 0.001$，$P = 0.001$，$P = 0.002$；$P = 0.002$，$P = 0.001$，$P = 0.001$）；药物干预开始后 7、14 d 时，蠲痹历节清方组和别嘌醇组滑膜中中性粒细胞数量均少于模型组（$P = 0.017$，$P = 0.014$；$P = 0.012$，$P = 0.014$）；药物干预开始后 21 d

时,对照组、蠲痹历节清方组及别嘌醇组滑膜中中性粒细胞数量均少于模型组($P=0.001$,$P=0.013$,$P=0.017$);其余各时点各组间两两比较,差异均无统计学意义。见表 5。



(1)A组　(2)B组　(3)C组　(4)D组

图 1　药物干预后 1 d 时 4 组动物踝关节滑膜组织形态(HE 染色　×250)



(1)A组　(2)B组　(3)C组　(4)D组

图 2　药物干预后 21 d 时 4 组动物踝关节滑膜组织形态(HE 染色　×250)

表 4　4 组动物药物干预开始后不同时点滑膜中血管数量的比较　$\bar{x}\pm s$,个

组别	样本量(只)	药物干预开始后时间点			
		1 d	7 d	14 d	21 d
对照组	10	4.30±0.20	4.50±0.30	4.40±0.40	4.50±0.70
模型组	10	9.60±1.50	11.20±1.40	13.20±1.10	15.40±2.20
蠲痹历节清方组	10	9.50±1.80	13.80±1.70	17.30±2.10	21.80±2.10
别嘌醇组	10	9.50±1.70	13.70±1.60	17.70±2.30	21.70±1.70
F 值		24.772	33.176	32.672	44.351
P 值		0.032	0.021	0.003	0.000

表 5　4 组动物药物干预开始后不同时点滑膜中中性粒细胞数量的比较　$\bar{x}\pm s$,个

组别	样本量(只)	药物干预开始后时间点			
		1 d	7 d	14 d	21 d
对照组	10	2.20±0.10	2.30±0.20	2.50±0.60	2.60±0.30
模型组	10	30.20±2.10	26.30±2.10	20.40±2.50	16.10±2.10
蠲痹历节清方组	10	30.70±1.60	22.40±3.20	11.30±2.30	4.30±1.10
别嘌醇组	10	30.90±1.70	23.10±2.30	11.90±2.20	4.60±2.30
F 值		32.347	43.561	42.361	51.745
P 值		0.001	0.001	0.014	0.011

4　讨　论

鸟类和部分灵长类动物与人体内嘌呤代谢的过程相似,代谢的最终产物为尿酸,因其体内的尿酸氧化酶(Urate Oxidase,UOX)基因存在无意突变而沉

默,无法合成 UOX 或者合成的 UOX 不具生物活性[5,11]。禽类嘌呤代谢过程与人类的代谢过程相似,其体内尿酸水平能够反映出体内嘌呤代谢的程度[7],其中鸡被认为是建立痛风模型的理想动物之一[11],我们也已在前期的研究中利用摄入高蛋白饮食和控制饮水成功复制出鸡急性痛风性关节炎模型[5]。造模结束时,除对照组外,其余 3 组动物均出现精神萎靡不振,闭目发呆,羽毛松乱、灰暗无光泽,有大面积脱毛,翅膀低垂,食欲减退、素囊空虚,鸡冠发白,双膝与双踝关节肿大,轻捏即躲闪哀鸣,跛行疼痛,站立不稳、喜伏卧,逐渐消瘦,粪便中白色物质增多等情况,血尿酸含量均大于 480 μmol · L^{-1},提示造模成功。

体内嘌呤代谢异常和(或)血尿酸排泄障碍,导致机体内血尿酸水平长期处于较高水平,尿酸沉积于关节软骨、滑膜引起疼痛和急性炎症反应是痛风发生的主要原因。黄嘌呤转化为尿酸是体内嘌呤代谢的最后一个环节,而 XOD 可催化体内的嘌呤底物形成尿酸,因此临床开发并应用 XOD 抑制剂治疗高尿酸血症及痛风[12]。目前临床上用于治疗痛风的 XOD 抑制剂较少,别嘌醇是其中应用较多的药物,但该药会引起皮疹、过敏反应和肾病等[13]。因此,在中草药中寻找安全有效的替代药物已成为目前药学研究的热点。

痛风属于中医学“历节”“痹症”范畴。对急性痛风性关节炎证素研究的结果表明,湿热是导致痛风性关节炎的最主要因素[14]。目前采用清热利湿方药治疗急性痛风性关节炎的临床研究也较多[15-16],但相关的作用机理尚未明确。蠲痹历节清方是湖南中医药大学第二附属医院治疗急性痛风性关节炎的内服方剂,经多年临床应用及观察,该方在缓解急性痛风性关节炎患者临床症状及降低血尿酸方面疗效确切。本方以清热燥湿药物为主,辅以活血止痛、通利关节药物。方中苍术健脾燥湿、祛风散寒,茵陈清热利湿,共为君药;黄柏、黄芩清热燥湿,土茯苓解毒除湿、通利关节,防己祛风湿、止痛、利水消肿,共为臣药;佐以当归活血止痛,泽泻利水渗湿、泄热,白术益气健脾、燥湿利水;甘草为使,缓急止痛、调和诸药。诸药合用,共奏健脾清热燥湿、活血通利止痛之功。

在本研究中,药物干预后蠲痹历节清方组动物一般状态及滑膜组织形态好于模型组,踝关节周径、血尿酸含量、血清 XOD 活性及滑膜中中性粒细胞数量均少于模型组,与别嘌醇组和对照组相当,提示蠲痹历节清方可通过抑制 XOD 活性,有效降低血尿酸水平,缓解鸡急性痛风性关节炎模型临床症状,其疗效与别嘌醇相当。

5 参考文献

[1] 熊辉,曲良烨,向黎黎,等. 痛风性关节炎湿热证病证结合模型的建立[J]. 中医正骨,2014,26(3):14 - 20.

[2] 尤力都孜·买买提,热比姑丽·伊斯拉木,阿布都吉力力·阿布都艾尼. 痛风及其动物模型研究初探[J]. 中药药理与临床,2012,28(3):172 - 173.

[3] 齐新宇,董大立,熊辉,等. 蠲痹历节清方治疗急性痛风性关节炎湿热证疗效观察[J]. 湖南中医药大学学报,2014,34(5):42 - 44.

[4] 齐新宇. 蠲痹历节清方对实验性鸡痛风模型血尿酸代谢的影响[D]. 长沙:湖南中医药大学,2014.

[5] 齐新宇,向黎黎,熊辉,等. 基于高蛋白饮食环境下痛风模型的量效研究[J]. 中医正骨,2015,27(2):1 - 6.

[6] 朱小甫,吴旭锦,郑义华. 高钙高蛋白日粮复制禽痛风病例研究[J]. 黑龙江畜牧兽医,2013,21(6):36 - 37.

[7] 任予勇. 实验性鸡高尿酸血症模型的建立[J]. 淮北职业技术学院学报,2013,12(5):133 - 135.

[8] 黄继汉,黄晓晖,陈志扬,等. 药理试验中动物间和动物与人体间的等效剂量换算[J]. 中国临床药理学与治疗学,2004,9(9):1069 - 1072.

[9] 匡红艳,程太平,胡建兵,等. 鸡持续高尿酸血症模型的制备方法研究[J]. 四川动物,2008,27(4):554 - 558.

[10] 苏友新,陈伟宏,王和鸣,等. 高尿酸血症及痛风性关节炎的动物模型研究概况[J]. 中国骨伤,2004,17(7):444 - 445.

[11] 楼秀余. 人类痛风病与 UOX 基因沉默相关的研究[J]. 中外医学研究,2012,10(29):154 - 155.

[12] 孔悦,张冰,刘小青,等. 黄嘌呤氧化酶活性在高尿酸血症动物模型中的变化[J]. 北京中医药大学学报,2004,27(6):38 - 40.

[13] 齐万虎,蒋企洲,蒋建勤. 黄瑞香中化学成分抑制黄嘌呤氧化酶活性及其机制研究[J]. 中国实验方剂学杂志,2014,20(5):141 - 144.

[14] 熊辉,姜国勇,胡楠,等. 痛风性关节炎中医证素及证型的聚类研究[J]. 中医正骨,2011,23(11):26 - 28.

[15] 吴小芳,刘书珍. 清热泻湿汤治疗急性痛风性关节炎 60 例观察与护理[J]. 实用中医药杂志,2014,30(4):278.

[16] 严立平,周正球. 清热利湿方治疗急性痛风性关节炎 38 例临床观察[J]. 吉林中医药,2012,32(6):600 - 601.

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