·基础研究·

补肾方对激素性股骨头坏死大鼠股骨头血管形态和血液状态的影响

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摘　要　目的:探讨补肾方对激素性股骨头坏死（osteonecroois of the femoral head,ONFH）大鼠股骨头血管形态和血液状态的影响。**方法:**将 120 只雄性 Wistar 大鼠随机分为空白组、模型组、健骨生丸组、补肾方高剂量组、补肾方中剂量组和补肾方低剂量组,每组 20 只。除空白组外,其余 5 组大鼠臀肌注射甲泼尼龙琥珀酸钠,连续注射 3 d,建立激素性 ONFH 模型。甲泼尼龙琥珀酸钠注射完后,空白组、模型组自由饮食;健骨生丸组按 1.68 g·kg^{-1}以健骨生丸灌胃,每天 1 次,连续 6 周;补肾方高、中、低剂量组分别按 21.2 g·kg^{-1}、10.6 g·kg^{-1}、5.3 g·kg^{-1}以补肾方灌胃,每天 1 次,连续 6 周。药物干预结束后,先从各组随机选取 10 只大鼠,麻醉后腹主动脉取血,动物死亡后,取双侧股骨头制成石蜡切片。将所取腹主动脉血分成 2 份,一份取血清测定甘油三酯（triglyceride,TG）、总胆固醇（total cholesterol,TC）、高密度脂蛋白（high density lipoprotein,HDL）、低密度脂蛋白（low density lipoprotein,LDL）、载脂蛋白 A1（apolipoprotein A1,ApoA1）及 ApoB;另一份取全血以旋转法测定全血黏度低切值、中切值和高切值,取血浆检测血浆黏度。制成的股骨头组织切片分成 2 份,一份进行 HE 染色,光学显微镜下观察骨组织形态;另一份进行免疫组织化学染色,测定血管内皮生长因子（vascular endothelial growth factor,VEGF）和 FLK1 蛋白表达情况。各组剩余的 10 只大鼠应用血管造影结合 Micro－CT 扫描技术测定股骨头中血管体积、血管表面积、血管体积分数及血管厚度。**结果:**①血脂检测结果。6 组大鼠血清 TG、TC、LDL、HDL、ApoA1 和 ApoB 含量比较,组间差异均有统计学意义（$F=4.538$,$P=0.004$;$F=3.322$,$P=0.018$;$F=2.681$,$P=0.043$;$F=2.621$,$P=0.047$;$F=2.400$,$P=0.035$;$F=3.741$,$P=0.010$）。空白组、健骨生丸组、补肾方中剂量组和补肾方高剂量组 TG、TC、LDL、ApoB 含量均低于模型组（$P=0.000$,$P=0.021$,$P=0.009$,$P=0.032$;$P=0.008$,$P=0.016$,$P=0.031$,$P=0.030$;$P=0.009$,$P=0.017$,$P=0.036$,$P=0.031$;$P=0.005$,$P=0.013$,$P=0.031$,$P=0.025$）,HDL、ApoA1 含量均高于模型组（$P=0.019$,$P=0.034$;$P=0.041$,$P=0.034$;$P=0.040$,$P=0.031$;$P=0.035$,$P=0.029$）;补肾方低剂量组 TC 含量低于模型组（$P=0.023$）,TG、LDL、HDL、ApoA1、ApoB 含量与模型组比较,组间差异均无统计学意义（$P=0.297$,$P=0.315$,$P=0.189$,$P=0.084$,$P=0.333$）;补肾方低剂量组血清 TG、TC、LDL、ApoB 含量均高于健骨生丸组（$P=0.037$,$P=0.018$,$P=0.041$,$P=0.047$）,HDL、ApoA1 含量均低于健骨生丸组（$P=0.046$,$P=0.043$）;补肾方中剂量组和健骨生丸组血清 TG、TC、LDL、HDL、ApoA1、ApoB 含量比较,组间差异均无统计学意义（$P=0.080$,$P=0.440$,$P=0.375$,$P=0.204$,$P=0.130$,$P=0.389$）;补肾方高剂量组血清 TG、TC、LDL、ApoB 含量均低于健骨生丸组（$P=0.019$,$P=0.022$,$P=0.024$,$P=0.039$）,HDL 含量高于健骨生丸组（$P=0.043$）,2 组 ApoA1 含量比较,差异无统计学意义（$P=0.094$）;补肾方中剂量组和高剂量组血清 TG、TC、LDL、ApoB 含量均低于低剂量组（$P=0.033$,$P=0.021$,$P=0.042$,$P=0.042$;$P=0.021$,$P=0.019$,$P=0.018$,$P=0.034$）,HDL 含量均高于低剂量组（$P=0.048$;$P=0.042$）,2 组 ApoA1 含量与补肾方低剂量组比较,组间差异均无统计学意义（$P=0.053$;$P=0.057$）;补肾方高剂量组血清 TG、TC、LDL、ApoB 含量均低于中剂量组（$P=0.029$,$P=0.020$,$P=0.020$,$P=0.035$）,HDL 含量高于中剂量组（$P=0.045$）,2 组 ApoA1 含量比较,组间差异无统计学意义（$P=0.239$）。②血液流变学指标检测结果。6 组大鼠全血黏度低切值、全血黏度中切值及血浆黏度比较,组间差异均有统计学意义（$F=3.291$,$P=0.019$;$F=3.256$,$P=0.020$;$F=3.779$,$P=0.010$）;6 组全血黏度高切值比较,差异无统计学意义（$F=2.460$,$P=0.059$）。空白组、健骨生丸组、补肾方中剂量组和补肾方高剂量组全血黏度低切值、全血黏度中切值及血浆黏度均低于模型组（$P=0.017$,$P=0.033$,$P=0.011$;$P=0.026$,$P=0.043$,$P=0.040$;$P=0.028$,$P=0.012$,$P=0.028$;$P=0.023$,$P=0.010$,$P=0.022$）;补肾方低剂量组全血黏度低切值、全血黏度中切值及血浆黏度与模型组比较,差异均无统计学意义（$P=0.085$,$P=0.069$,$P=0.094$）;补肾方低剂量组血浆黏度高于健骨生丸组（$P=0.049$）,2 组全血黏度低切值、全血黏度中切值比较,组间差异均无统计学意义（$P=0.054$,$P=0.057$）;补肾方中剂量组和健骨生丸组全血黏度低切值、全血黏度中切值及血浆黏度比较,组间差异均无统计学意义（$P=0.091$,$P=0.083$,$P=0.055$）;补肾方高剂量组全血黏度中切值和血浆黏度

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均低于健骨生丸组($P=0.045$,$P=0.014$),2 组全血黏度低切值比较,差异无统计学意义($P=0.214$);补肾方中剂量组全血黏度低切值、血浆黏度均低于低剂量组($P=0.048$,$P=0.032$),2 组全血黏度中切值比较,组间差异均无统计学意义($P=0.051$);补肾方高剂量组全血黏度低切值、全血黏度中切值及血浆黏度均低于低剂量组($P=0.030$,$P=0.048$,$P=0.013$);补肾方高剂量组全血黏度低切值、血浆黏度均低于中剂量组($P=0.049$,$P=0.027$),2 组全血黏度中切值比较,组间差异无统计学意义($P=0.052$)。③VEGF 蛋白和 FLK1 蛋白测定结果。6 组大鼠股骨头内 VEGF 蛋白和 FLK1 蛋白表达量比较,组间差异均有统计学意义($F=9.519$,$P=0.000$;$F=5.317$,$P=0.009$)。空白组、健骨生丸组、补肾方中剂量组和补肾方高剂量组 VEGF 蛋白和 FLK1 蛋白表达量均高于模型组($P=0.000$,$P=0.000$;$P=0.005$,$P=0.009$;$P=0.004$,$P=0.008$;$P=0.000$,$P=0.000$);补肾方低剂量组 VEGF 蛋白表达量与模型组比较,差异无统计学意义($P=0.051$),FLK1 蛋白表达量高于模型组($P=0.047$);补肾方低剂量组 VEGF 蛋白和 FLK1 蛋白表达量均低于健骨生丸组($P=0.041$,$P=0.036$);补肾方中剂量组 VEGF 蛋白和 FLK1 蛋白表达量与健骨生丸组比较,组间差异均无统计学意义($P=0.175$;$P=0.221$);补肾方高剂量组 VEGF 蛋白和 FLK1 蛋白表达量均高于健骨生丸组($P=0.045$;$P=0.047$);补肾方中剂量组和高剂量组 VEGF 蛋白、FLK1 蛋白表达量均高于低剂量组($P=0.047$,$P=0.044$;$P=0.016$,$P=0.011$);补肾方高剂量组 FLK1 蛋白表达量高于中剂量组($P=0.042$),2 组 VEGF 蛋白表达量比较,组间差异无统计学意义($P=0.051$)。④股骨头内血管 Micro-CT 检查结果。6 组大鼠股骨头血管体积、血管表面积、血管体积分数、血管厚度比较,组间差异均有统计学意义($F=36.442$,$P=0.000$;$F=7.080$,$P=0.000$;$F=27.869$,$P=0.000$;$F=8.371$,$P=0.000$)。空白组、健骨生丸组、补肾方中剂量组、补肾方高剂量组血管体积、血管表面积、血管体积分数、血管厚度均大于模型组($P=0.000$,$P=0.000$,$P=0.000$,$P=0.000$;$P=0.009$,$P=0.003$,$P=0.002$,$P=0.001$;$P=0.000$,$P=0.001$,$P=0.001$,$P=0.000$;$P=0.007$,$P=0.015$,$P=0.011$,$P=0.005$);补肾方低剂量组与模型组血管体积、血管表面积、血管体积分数、血管厚度比较,组间差异均无统计学意义($P=0.051$,$P=0.052$,$P=0.082$,$P=0.064$);补肾方低剂量组、补肾方中剂量组与健骨生丸组血管体积、血管表面积、血管体积分数、血管厚度比较,组间差异均无统计学意义($P=0.057$,$P=0.063$,$P=0.051$,$P=0.052$;$P=1.000$,$P=0.222$,$P=1.000$,$P=0.813$);补肾方高剂量组血管体积、血管表面积、血管体积分数、血管厚度均大于健骨生丸组($P=0.000$,$P=0.017$,$P=0.000$,$P=0.010$);补肾方中剂量组和高剂量组血管体积、血管表面积、血管体积分数、血管厚度均大于低剂量组($P=0.000$,$P=0.023$,$P=0.001$,$P=0.021$;$P=0.000$,$P=0.015$,$P=0.000$,$P=0.007$);补肾方高剂量组血管体积、血管表面积、血管体积分数、血管厚度均大于中剂量组($P=0.000$,$P=0.019$,$P=0.000$,$P=0.009$)。**结论**:补肾方能促进激素性 ONFH 大鼠股骨血管修复,改善股骨头血液微循环状态,其作用可能与补肾方增加股骨头内 VEGF 和 FLK1 蛋白表达有关,且中剂量补肾方的疗效与健骨生丸相当,高剂量补肾方的疗效优于健骨生丸。

关键词　股骨头坏死;大鼠,Wistar;补肾方;血管形态;血液状态;动物实验

Effect of Bushen Fang(补肾方)on blood vessel morphous of femoral head and blood state in rats with steroid-induced necrosis of femoral head

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ABSTRACT　**Objective**:To explore the effect of Bushen Fang(补肾方,BSF)on blood vessel morphous of femoral head and blood state in rats with steroid-induced necrosis of femoral head(SNFH). **Methods**:One hundred and twenty Wistar male rats were randomly divided into blank group,model group,Jiangusheng Wan(健骨生丸,JGSW)group,BSF high-dose group,BSF middle-dose group and BSF low-dose group,20 cases in each group. The rats in model group,JGSW group,BSF high-dose group,BSF middle-dose group and BSF low-dose group were treated with intra-gluteal injection of methylprednisolone sodium succinate for consecutive 3 days to build the SNFH models. Then the rats in blank group and model group were permitted to drink and eat freely. The rats in JGSW group were intragastric administrated with JGSW(1.68 g/kg),once a day for consecutive 6 weeks;while the others in BSF high-dose group,BSF middle-dose group and BSF low-dose group were intragastric administrated with BSF(21.2,10.6 and 5.3 g/kg,respectively),once a day for consecutive 6 weeks. After the end of drug intervention,10 rats were randomly selected from each group,and their blood were fetched out from aorta abdominalis after anesthesia and the bilateral femoral heads were fetched out for making paraffin sections after the rats died. The blood were divided into 2 parts,and

one of them were applied to measure the serum contents of triglyceride(TG),total cholesterol(TC),high density lipoprotein(HDL),low density lipoprotein(LDL),apolipoprotein A1(ApoA1) and ApoB;while the other portion were applied to measure the high,medium and low shear whole blood viscosity and the plasma viscosity. The acquired tissue sections of femoral head were divided into 2 parts,and one of them were received HE staining for observing the bone tissue morphology under optical microscope,while the other portion were received immunohistochemical staining for detecting the expression of vascular endothelial growth factor(VEGF) protein and FLK1 protein. The remaining 10 rats in each group were used to measure the volume, surface area,volume fraction and thickness of blood vessel in femoral head by using angiography combined with Micro-CT scanning technique. **Results**:There was statistical difference in serum content of TG,TC,LDL,HDL,ApoA1 and ApoB between the 6 groups($F=4.538$,$P=0.004$;$F=3.322$,$P=0.018$;$F=2.681$,$P=0.043$;$F=2.621$,$P=0.047$;$F=2.400$,$P=0.035$;$F=3.741$,$P=0.010$). The serum content of TG,TC,LDL and ApoB were lower and the serum content of HDL and ApoA1 were higher in blank group,JGSW group,BSF middle-dose group and BSF high-dose group compared to model group($P=0.000$,$P=0.021$,$P=0.009$,$P=0.032$;$P=0.008$,$P=0.016$,$P=0.031$,$P=0.030$;$P=0.009$,$P=0.017$,$P=0.036$,$P=0.031$;$P=0.005$,$P=0.013$,$P=0.031$,$P=0.025$;$P=0.019$,$P=0.034$;$P=0.041$,$P=0.034$;$P=0.040$,$P=0.031$;$P=0.035$,$P=0.029$). The serum content of TC were lower in BSF low-dose group compared to model group($P=0.023$),and there was no statistical difference in serum content of TG,LDL,HDL,ApoA1 and ApoB between BSF low-dose group and model group($P=0.297$,$P=0.315$,$P=0.189$,$P=0.084$,$P=0.333$). The serum content of TG,TC,LDL and ApoB were higher and the serum content of HDL and ApoA1 were lower in BSF low-dose group compared to JGSW group($P=0.037$,$P=0.018$,$P=0.041$,$P=0.047$,$P=0.046$,$P=0.043$). There was no statistical difference in serum content of TG,TC,LDL,HDL,ApoA1 and ApoB between BSF middle-dose group and JGSW group($P=0.080$,$P=0.440$,$P=0.375$,$P=0.204$,$P=0.130$,$P=0.389$). The serum content of TG,TC,LDL and ApoB were lower and the serum content of HDL was higher in BSF high-dose group compared to JGSW group($P=0.019$,$P=0.022$,$P=0.024$,$P=0.039$,$P=0.043$),and there was no statistical difference in serum content of ApoA1 between the two groups($P=0.094$). The serum content of TG,TC,LDL and ApoB were lower and the serum content of HDL was higher in BSF middle-dose group and high-dose group compared to low-dose group($P=0.033$,$P=0.021$,$P=0.042$,$P=0.042$;$P=0.021$,$P=0.019$,$P=0.018$,$P=0.034$;$P=0.048$;$P=0.042$),and there was no statistical difference in serum content of ApoA1 between BSF middle-dose group and BSF low-dose group and between BSF high-dose group and BSF low-dose group($P=0.053$;$P=0.057$). The serum content of TG,TC,LDL and ApoB were lower and the serum content of HDL was higher in BSF high-dose group compared to BSF middle-dose group($P=0.029$,$P=0.020$,$P=0.020$,$P=0.035$,$P=0.045$),and there was no statistical difference in serum content of ApoA1 between the two groups($P=0.239$). The detection results of hemorheological indexs showed that there was statistical difference in low and medium shear whole blood viscosity and plasma viscosity between the 6 groups($F=3.291$,$P=0.019$;$F=3.256$,$P=0.020$;$F=3.779$,$P=0.010$),and there was no statistical difference in the high shear whole blood viscosity between the 6 groups($F=2.460$,$P=0.059$). The low and medium shear whole blood viscosity and plasma viscosity were lower in blank group,JGSW group,BSF middle-dose group and BSF high-dose group compared to model group($P=0.017$,$P=0.033$,$P=0.011$;$P=0.026$,$P=0.043$,$P=0.040$;$P=0.028$,$P=0.012$,$P=0.028$;$P=0.023$,$P=0.010$,$P=0.022$). There was no statistical difference in low and medium shear whole blood viscosity and plasma viscosity between BSF low-dose group and model group($P=0.085$,$P=0.069$,$P=0.094$). The plasma viscosity was higher in BSF low-dose group compared to JGSW group($P=0.049$),and there was no statistical difference in low and medium shear whole blood viscosity between the two groups($P=0.054$,$P=0.057$). There was no statistical difference in low and medium shear whole blood viscosity and plasma viscosity between BSF middle-dose group and JGSW group($P=0.091$,$P=0.083$,$P=0.055$). The medium shear whole blood viscosity and plasma viscosity were lower in BSF high-dose group compared to JGSW group($P=0.045$,$P=0.014$),and there was no statistical difference in the low shear whole blood viscosity between the two groups($P=0.214$). The low shear whole blood viscosity and plasma viscosity were lower in BSF middle-dose group compared to BSF low-dose group($P=0.048$,$P=0.032$),and there was no statistical difference in the medium shear whole blood viscosity between the two groups($P=0.051$). The low and medium shear whole blood viscosity and plasma viscosity were lower in BSF high-dose group compared to BSF low-dose group($P=0.030$,$P=0.048$,$P=0.013$). The low shear whole blood viscosity and plasma viscosity were lower in BSF high-dose group compared to BSF middle-dose group($P=0.049$,$P=0.027$),and there was no statistical difference in the medium shear whole blood viscosity between the two groups($P=0.052$). There was statistical difference in the expression of VEGF protein and FLK1 protein in femoral heads between the 6 groups($F=9.519$,$P=0.000$;$F=5.317$,$P=0.009$). The VEGF and FLK1 protein expression were higher in blank group,

JGSW group, BSF middle-dose group and BSF high-dose group compared to model group($P=0.000$, $P=0.000$; $P=0.005$, $P=0.009$; $P=0.004$, $P=0.008$; $P=0.000$, $P=0.000$). There was no statistical difference in the VEGF protein expression between BSF low-dose group and model group($P=0.051$), while the FLK1 protein expression were higher in BSF low-dose group comared to model group($P=0.047$). The VEGF and FLK1 protein expression were lower in BSF low-dose group compared to JGSW group($P=0.041$, $P=0.036$). There was no statistical difference in the VEGF and FLK1 protein expression between BSF middle-dose group and JGSW group($P=0.175$; $P=0.221$). The VEGF and FLK1 protein expression were higher in BSF high-dose group compared to JGSW group($P=0.045$; $P=0.047$). The VEGF and FLK1 protein expression were higher in BSF middle-dose group and high-dose group compared to low-dose group($P=0.047$, $P=0.044$; $P=0.016$, $P=0.011$). The FLK1 protein expression was higher in BSF high-dose group compared to middle-dose group($P=0.042$), and there was no statistical difference in the VEGF protein expression between the two groups($P=0.051$). The results of Micro-CT examination showed that there was statistical difference in the volumes, surface area, volume fraction and thickness of blood vessels in femoral head between the 6 groups($F=36.442$, $P=0.000$; $F=7.080$, $P=0.000$; $F=27.869$, $P=0.000$; $F=8.371$, $P=0.000$). The volumes, surface area, volume fraction and thickness of blood vessels were larger in blank group, JGSW group, BSF middle-dose group and BSF high-dose group compared to model group($P=0.000$, $P=0.000$, $P=0.000$, $P=0.000$; $P=0.009$, $P=0.003$, $P=0.002$, $P=0.001$; $P=0.000$, $P=0.001$, $P=0.001$, $P=0.000$; $P=0.007$, $P=0.015$, $P=0.011$, $P=0.005$). There was no statistical difference in the volumes, surface area, volume fraction and thickness of blood vessels between BSF low-dose group and model group($P=0.051$, $P=0.052$, $P=0.082$, $P=0.064$). There was no statistical difference in the volumes, surface area, volume fraction and thickness of blood vessels between BSF low-dose group and JGSW group and between BSF middle-dose group and JGSW group($P=0.057$, $P=0.063$, $P=0.051$, $P=0.052$; $P=1.000$, $P=0.222$, $P=1.000$, $P=0.813$). The volumes, surface area, volume fraction and thickness of blood vessels were larger in BSF high-dose group compared to JGSW group($P=0.000$, $P=0.017$, $P=0.000$, $P=0.010$). The volumes, surface area, volume fraction and thickness of blood vessels were larger in BSF middle-dose group and high-dose group compared to low-dose group($P=0.000$, $P=0.023$, $P=0.001$, $P=0.021$; $P=0.000$, $P=0.015$, $P=0.000$, $P=0.007$). The volumes, surface area, volume fraction and thickness of blood vessels were larger in BSF high-dose group compared to middle-dose group($P=0.000$, $P=0.019$, $P=0.000$, $P=0.009$). **Conclusion**: Application of BSF can promote femoral blood vessel repair and improve blood microcirculation of femoral head in rats with SNFH, and the effect may be related to the increase of expression of VEGF protein and FLK1 protein in the femoral head. The middle-dose BSF is similar to JGSW while the high-dose BSF surpasses JGSW in the curative effect.

Key words　femur head necrosis; rats, wistar; Bushen Fang; morphous of blood vessel; state of blood; animal experimentation

激素性股骨头坏死(osteonecroois of the femoral head,ONFH)的发病率居非创伤性 ONFH 的首位[1],其发病机制尚不完全清楚[2]。补肾方具有补肾壮骨、活血通络的功效,是临床治疗非创伤性 ONFH 的验效方之一[3]。有研究显示,该方可促进激素性 ONFH 鸡坏死股骨头成骨、抑制骨破坏,但其是否能修复坏死区的血管目前尚不清楚。因此,本研究拟通过动物实验探讨补肾方对激素性 ONFH 血管的修复作用,进一步研究其治疗 ONFH 的作用机制。

1　材料与仪器

1.1　实验动物　雄性 12 周龄 SPF 级 Wistar 大鼠 120 只,体质量 300 ~ 320 g,由军事医学科学院实验动物中心提供,动物合格证号 SCXK -(军)2007 -004。实验方案通过医学动物实验伦理委员会批准。

1.2　药物和试剂　补肾方浸膏(中国中医科学院中药研究所),药物组成包括熟地黄 20 g、山药 12 g、山茱萸 12 g、肉桂 6 g、杜仲 10 g、鹿角胶(烊化)20 g、当归 10 g、菟丝子 15 g、狗脊 15 g;健骨生丸(北京匡达制药厂,批号:国药准字 Z10970030);注射用甲泼尼龙琥珀酸钠(Pfizer Manufacturing Belgium NV,批号:H20080285);水合氯醛(国药集团化学试剂有限公司,批号:T20080530);多聚甲醛(北京益利精细化学品有限公司,批号:20070407);甘油三酯(triglyceride,TG)试剂盒、总胆固醇(total cholesterol,TC)试剂盒、高密度脂蛋白(high density lipoprotein,HDL)试剂盒、低密度脂蛋白(low density lipoprotein,LDL)试剂盒(湖南永和阳光科技有限责任公司);载脂蛋白 A1(apolipoprotein A1,ApoA1)试剂盒、APoB 试剂盒(北京利德曼公司);肝素(北京华英生物技术研究所);兔多克隆抗血管内皮生长因子(vascular endothelial growth factor,VEGF)抗体(Abcam);FLK1 抗体(Cell Signaling Tech);MV -122 Microfill 造影剂(Flow

Tech）。

1.3 实验仪器 SKY SCAN－1172 MICRO－CT（BRUKER microCT），BH－2 光学显微镜（OLYMPUS），BMJ－1 生物组织包埋机、QPJ－C 轮转式切片机（天津天利航空机电有限公司），BT－300 博莱特血流变仪（博莱特通有限公司），LG－PABER－Ⅰ血小板聚集凝血因子分析仪（北京世带科学仪器公司），血液黏度仪（北京中勤世帝科学仪器有限公司）。

2 方 法

2.1 分组及造模 适应性喂养 1 周后将 120 只大鼠随机分为空白组、模型组、健骨生丸组、补肾方高剂量组、补肾方中剂量组和补肾方低剂量组，每组 20 只。除空白组外，其余 5 组大鼠按 21 mg·kg^{-1}臀肌注射甲泼尼龙琥珀酸钠，每天 1 次，连续 3 d，建立激素性 ONFH 模型[4-8]。所有动物均给予标准化饮食并不限制其活动。

2.2 药物干预 甲泼尼龙琥珀酸钠注射完后，空白组、模型组自由饮食；健骨生丸组按 1.68 g·kg^{-1}以健骨生丸灌胃，每天 1 次，连续 6 周；补肾方高、中、低剂量组分别按 21.2 g·kg^{-1}、10.6 g·kg^{-1}、5.3 g·kg^{-1}补肾方灌胃，每天 1 次，连续 6 周。

2.3 实验观察 药物干预结束后，先从各组随机选取 10 只大鼠，麻醉后腹主动脉取血，动物死亡后，取双侧股骨头置于 4% 多聚甲醛中固定 72 h，经脱钙、石蜡包埋后制成厚度为 4 μm 的切片。将所取腹主动脉血分成 2 份，一份取血清测定 TG、TC、HDL、LDL、ApoA1、ApoB；另一份取全血以旋转法测定全血黏度低切值、全血黏度中切值、全血黏度高切值，取血浆检测血浆黏度。制成的股骨头组织切片分成 2 份，一份进行 HE 染色，光学显微镜下观察骨组织形态；另一份切片依次经脱蜡至水、3% 双氧水处理、蒸馏水洗涤、37 ℃复合酶消化、PBS 洗涤、血清封闭、滴加一抗（浓度 1∶50）、4 ℃条过夜处理后，用即用型 SABC 检测试剂盒进行股骨头内 VEGF 和 FLK1 免疫组织化学染色，应用 IPP 图像处理分析系统计阳性细胞百分数。

将各组剩余的 10 只大鼠用 10% 水合氯醛溶液按 1 mL·kg^{-1}腹腔注射麻醉，自左心室插入灌注管到升主动脉，灌注 500 mL 生理盐水后，用多聚甲醛灌注进行组织和血管固定，将造影剂推入血管，大鼠置于 4 ℃冰箱过夜，第 2 天取股骨头标本置入 4% 多聚甲醛溶液固定，用 10% EDTA－Na2 溶液脱钙后，采用 SKY SCAN－1172 MICRO－CT 进行股骨头扫描，扫描参数：二值化阈值 72～255、电压 75 kV、电流 133 mA、兴趣区域 6.88 μm×6.88 μm。用 CTA V1.1.13软件进行三维重建，并通过分析软件计算血管体积、血管表面积、血管体积分数及血管厚度。

2.4 数据统计分析 采用 SPSS 17.0 软件进行数据统计分析，6 组大鼠血脂指标（TG、TC、HDL、LDL、ApoA1、ApoB）、血液流变学指标（全血黏度低切值、全血黏度中切值、全血黏度高切值、血浆黏度）、血管参数（血管体积、血管表面积、血管体积分数、血管厚度）总体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 血脂检测结果 6 组大鼠血清 TG、TC、LDL、HDL、ApoA1 和 ApoB 含量比较，差异均有统计学意义。空白组、健骨生丸组、补肾方中剂量组和补肾方高剂量组 TG、TC、LDL、ApoB 含量均低于模型组（$P=0.000$，$P=0.021$，$P=0.009$，$P=0.032$；$P=0.008$，$P=0.016$，$P=0.031$，$P=0.030$；$P=0.009$，$P=0.017$，$P=0.036$，$P=0.031$；$P=0.005$，$P=0.013$，$P=0.031$，$P=0.025$），HDL、ApoA1 含量均高于模型组（$P=0.019$，$P=0.034$；$P=0.041$，$P=0.034$；$P=0.040$，$P=0.031$；$P=0.035$，$P=0.029$）；补肾方低剂量组 TC 含量低于模型组（$P=0.023$），TG、LDL、HDL、ApoA1、ApoB 含量与模型组比较，组间差异均无统计学意义（$P=0.297$，$P=0.315$，$P=0.189$，$P=0.084$，$P=0.333$）；补肾方低剂量组血清 TG、TC、LDL、ApoB 含量均高于健骨生丸组（$P=0.037$，$P=0.018$，$P=0.041$，$P=0.047$），HDL、ApoA1 含量均低于健骨生丸组（$P=0.046$，$P=0.043$）；补肾方中剂量组和健骨生丸组血清 TG、TC、LDL、HDL、ApoA1、ApoB 含量比较，组间差异均无统计学意义（$P=0.080$，$P=0.440$，$P=0.375$，$P=0.204$，$P=0.130$，$P=0.389$）；补肾方高剂量组血清 TG、TC、LDL、ApoB 均含量均低于健骨生丸组（$P=0.019$，$P=0.022$，$P=0.024$，$P=0.039$），HDL 含量高于健骨生丸组（$P=0.043$），2 组 ApoA1 含量比较，差异无统计学意义（$P=0.094$）；补肾方中剂量组和高剂量组血清 TG、TC、LDL、ApoB 含量均低于低剂量组（$P=0.033$，$P=0.021$，$P=0.042$，$P=0.042$；$P=0.021$，$P=0.019$，

$P=0.018$，$P=0.034$），HDL 含量均高于低剂量组（$P=0.048$；$P=0.042$），2 组 ApoA1 含量与补肾方低剂量组比较，组间差异均无统计学意义（$P=0.053$；$P=0.057$）；补肾方高剂量组血清 TG、TC、LDL、ApoB 含量均低于中剂量组（$P=0.029$，$P=0.020$，$P=0.020$，$P=0.035$），HDL 含量高于中剂量组（$P=0.045$），2 组 ApoA1 含量比较，组间差异无统计学意义（$P=0.239$）。见表 1。

表 1　6 组大鼠血脂检测结果　$\bar{x}\pm s$

组别	样本量（只）	TG（mmol·L^{-1}）	TC（mmol·L^{-1}）	LDL（mmol·L^{-1}）	HDL（mmol·L^{-1}）	ApoA1（g·L^{-1}）	ApoB（g·L^{-1}）
空白组	10	0.15±0.04	1.11±0.32	0.34±0.12	0.88±0.24	1.30±0.44	1.19±0.42
模型组	10	0.56±0.18	1.89±0.58	0.91±0.26	0.36±0.16	1.00±0.41	2.36±0.40
健骨生丸组	10	0.48±0.11	1.79±0.39	0.84±0.18	0.44±0.16	1.29±0.10	2.25±0.52
补肾方低剂量组	10	0.52±0.15	1.82±0.49	0.89±0.24	0.39±0.17	1.22±0.25	2.31±0.43
补肾方中剂量组	10	0.47±0.11	1.76±0.38	0.82±0.19	0.43±0.12	1.28±0.17	2.23±0.57
补肾方高剂量组	10	0.32±0.07	1.45±0.42	0.63±0.12	0.58±0.19	1.28±0.21	1.86±0.48
F 值		4.538	3.322	2.681	2.621	2.400	3.741
P 值		0.004	0.018	0.043	0.047	0.035	0.010

3.2　血液流变学指标检测结果　6 组大鼠全血黏度低切值、全血黏度中切值及血浆黏度比较，差异均有统计学意义；6 组全血黏度高切值比较，差异无统计学意义。空白组、健骨生丸组、补肾方中剂量组和补肾方高剂量组全血黏度低切值、全血黏度中切值及血浆黏度均低于模型组（$P=0.017$，$P=0.033$，$P=0.011$；$P=0.026$，$P=0.043$，$P=0.040$；$P=0.028$，$P=0.012$，$P=0.028$；$P=0.023$，$P=0.010$，$P=0.022$）；补肾方低剂量组全血黏度低切值、全血黏度中切值及血浆黏度与模型组比较，差异均无统计学意义（$P=0.085$，$P=0.069$，$P=0.094$）；补肾方低剂量组血浆黏度高于健骨生丸组（$P=0.049$），2 组全血黏度低切值、全血黏度中切值比较，组间差异均无统计学意义（$P=0.054$，$P=0.057$）；补肾方中剂量组和健骨生丸组全血黏度低切值、全血黏度中切值及血浆黏度比较，组间差异均无统计学意义（$P=0.091$，$P=0.083$，$P=0.055$）；补肾方高剂量组全血黏度中切值和血浆黏度均低于健骨生丸组（$P=0.045$，$P=0.014$），2 组全血黏度低切值比较，差异无统计学意义（$P=0.214$）；补肾方中剂量组全血黏度低切值、血浆黏度均低于低剂量组（$P=0.048$，$P=0.032$），2 组全血黏度中切值比较，组间差异均无统计学意义（$P=0.051$）；补肾方高剂量组全血黏度低切值、全血黏度中切值及血浆黏度均低于低剂量组（$P=0.030$，$P=0.048$，$P=0.013$）；补肾方高剂量组全血黏度低切值、血浆黏度均低于中剂量组（$P=0.049$，$P=0.027$），2 组全血黏度中切值比较，组间差异无统计学意义（$P=0.052$）。见表 2。

3.3　股骨头组织学观察结果　股骨头组织 HE 染色结果显示，空白组大鼠股骨头骨小梁宽度正常，未见狭窄或断裂，周围可见成骨细胞和少量破骨细胞，骨细胞内空骨陷窝和核固缩较少，骨髓腔内各种血细胞丰富，未见坏死区，脂肪细胞未见增多和变大；模型组大鼠股骨头骨小梁变窄或断裂，骨细胞内可见大量空骨陷窝和核固缩，骨髓腔内血细胞减少，可见大量坏死区，脂肪细胞明显增多变大；健骨生丸组和补肾方高、中、低剂量组股骨头坏死病理变化较模型组减轻，关节软骨、骨小梁的结构和形态改善，脂肪细胞减少。见图 1。

6 组大鼠股骨头内 VEGF 蛋白和 FLK1 蛋白表达量比较，差异均有统计学意义。空白组、健骨生丸组、补肾方中剂量组和补肾方高剂量组 VEGF 蛋白和 FLK1 蛋白表达量均高于模型组（$P=0.000$，$P=0.000$；$P=0.005$，$P=0.009$；$P=0.004$，$P=0.008$；$P=0.000$，$P=0.000$）；补肾方低剂量组 VEGF 蛋白表达量与模型组比较，差异无统计学意义（$P=0.051$），FLK1 蛋白表达量高于模型组（$P=0.047$）；补肾方低剂量组 VEGF 蛋白和 FLK1 蛋白表达量均低于健骨生丸组（$P=0.041$，$P=0.036$）；补肾方中剂量组 VEGF 蛋白和 FLK1 蛋白表达量与健骨生丸组比较，组间差异均无统计学意义（$P=0.175$；$P=0.221$）；补肾方高剂量组 VEGF 蛋白和 FLK1 蛋白表达量均高于健骨生丸组（$P=0.045$；$P=0.047$）；补肾方中剂量组和高剂量组 VEGF 蛋白、FLK1 蛋白表达

量均高于低剂量组（$P=0.047$，$P=0.044$；$P=0.016$，$P=0.011$）；补肾方高剂量组 FLK1 蛋白表达量高于中剂量组（$P=0.042$），2 组 VEGF 蛋白表达量比较，组间差异无统计学意义（$P=0.051$）。见图 2、图 3、表 2。

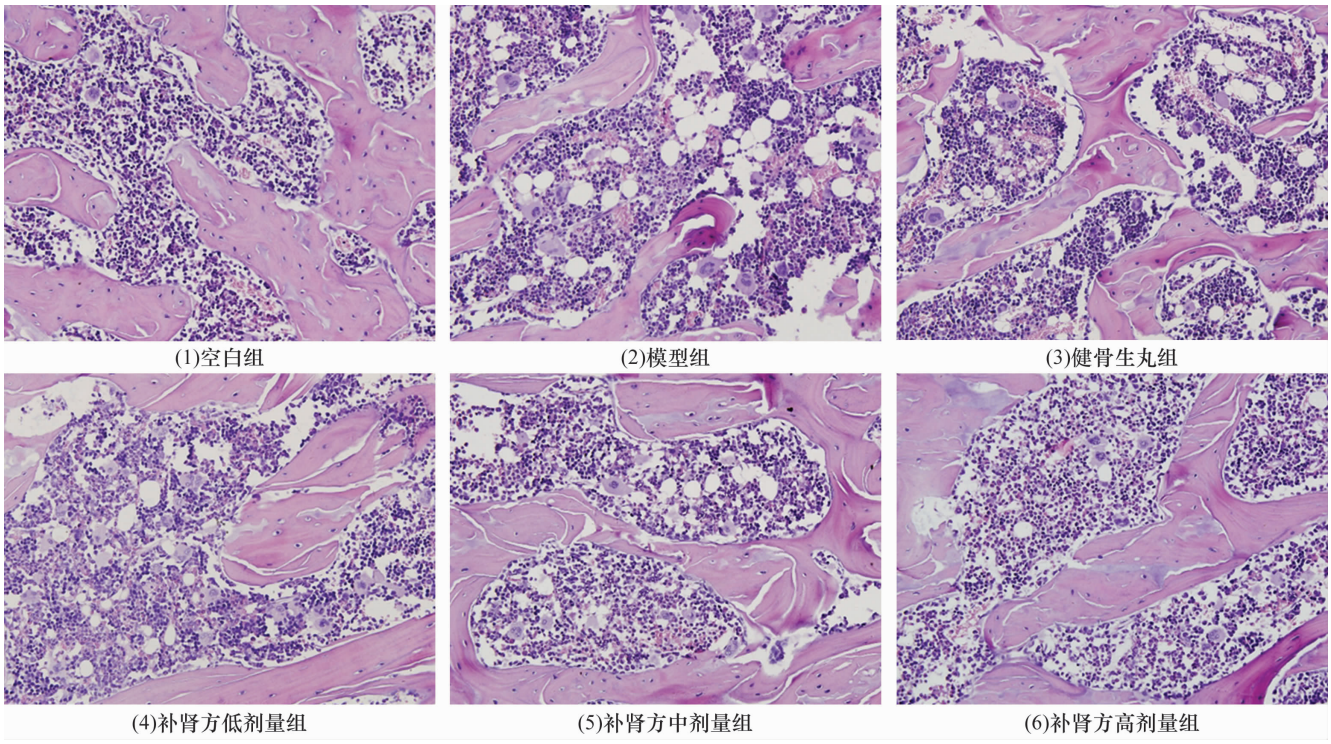

图 1　6 组大鼠股骨头组织学观察结果（HE 染色　×200）

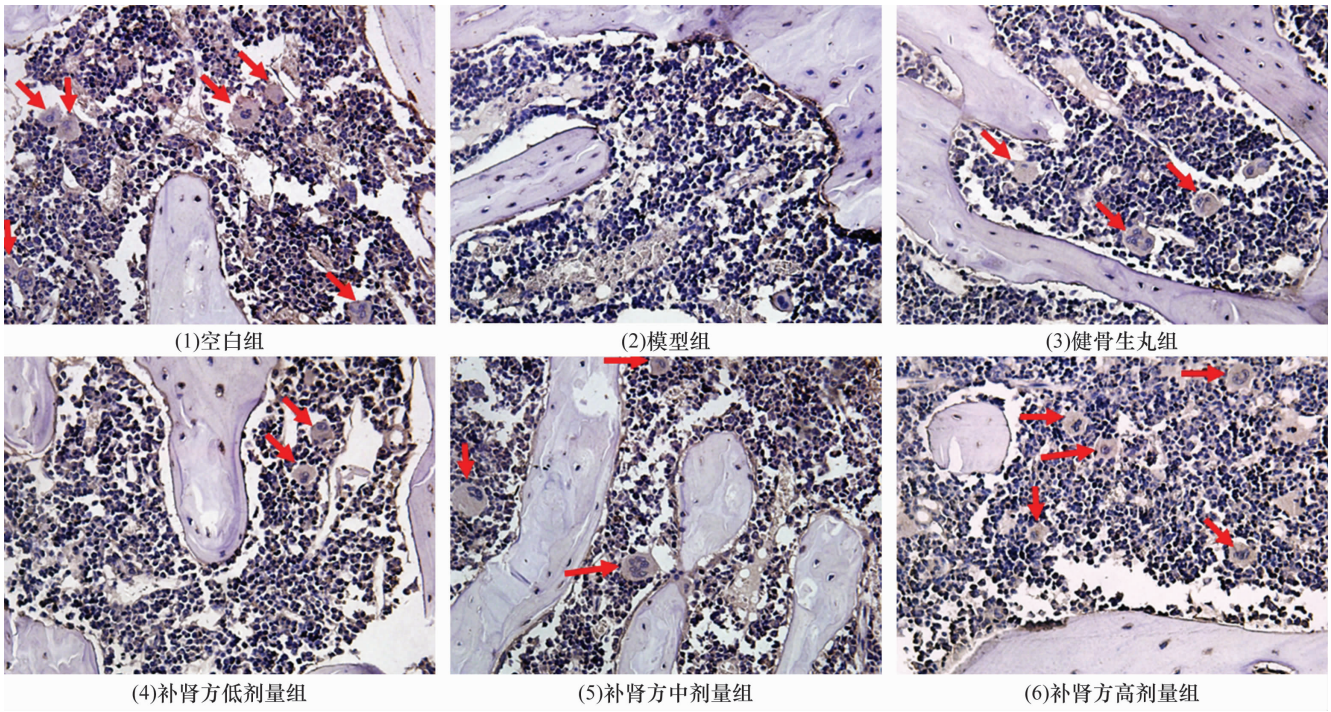

图 2　6 组大鼠股骨头组织 VEGF 表达情况（免疫组化染色　×200）

3.4　股骨头内血管 Micro－CT 检查结果　空白组大鼠股骨头内血管微结构清晰，血管丰富成网状，粗细均匀，无明显断裂；模型组大鼠股骨头内血管微结构模糊不清，血管较少不成网状，与空白组相比明显变细减少且有断裂；健骨生丸组和补肾方中、高剂量组血管微结构较清晰，血管成网状；补肾方低剂量组血管微结构模糊，血管较少不成网状（图 4）。

6 组大鼠股骨头血管体积、血管表面积、血管体积分数、血管厚度比较，差异均有统计学意义。空白组、健骨生丸组、补肾方中剂量组、补肾方高剂量组血

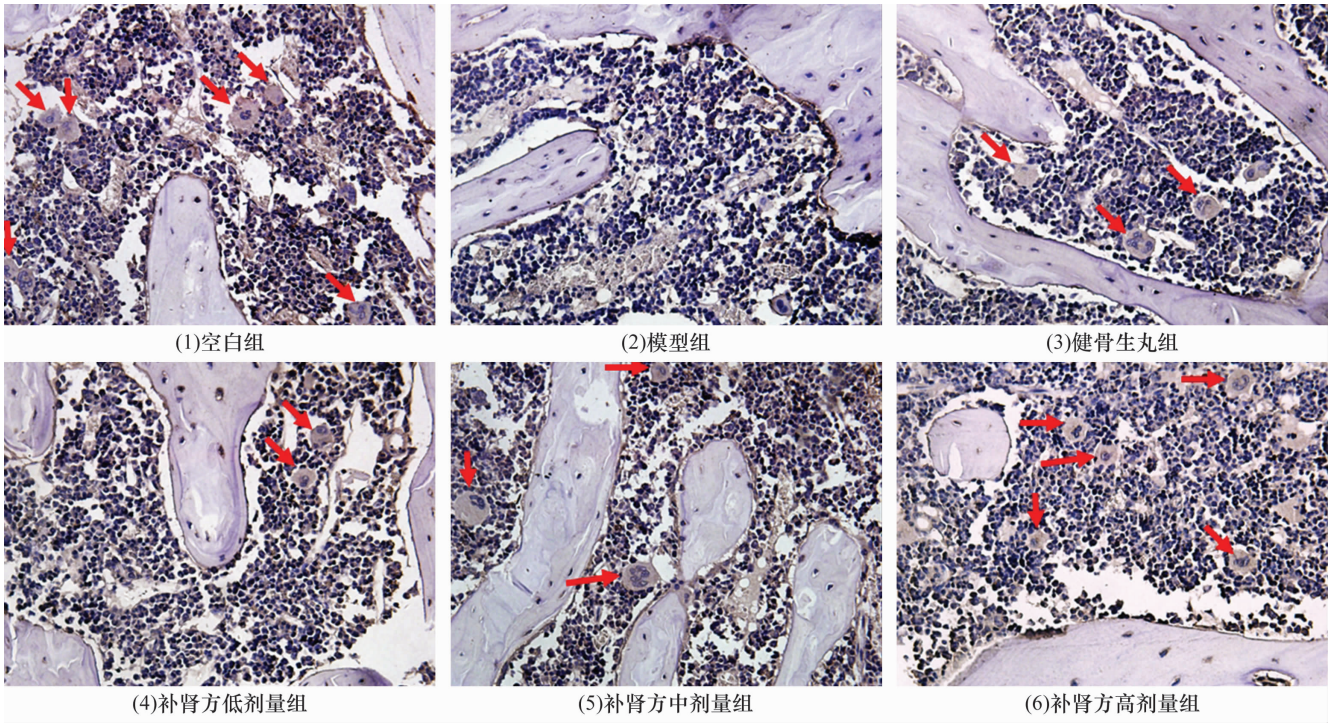

图 3　6 组大鼠股骨头组织 FLK1 表达情况（免疫组化染色　×200）

表 2　6 组大鼠血液流变学指标检测结果及 VEGF、FLK1 蛋白表达量　$\bar{x} \pm s$

组别	样本量（只）	全血黏度（mpa·s）			血浆黏度（mpa·s）	VEGF（%）	FLK1（%）
		低切值	中切值	高切值			
空白组	10	26.41 ± 7.34	12.50 ± 7.34	4.82 ± 1.54	1.05 ± 0.23	57.61 ± 11.4	82.16 ± 8.47
模型组	10	47.12 ± 9.89	29.48 ± 7.89	7.33 ± 1.39	1.90 ± 0.40	16.52 ± 3.40	20.37 ± 6.43
健骨生丸组	10	41.31 ± 10.93	27.84 ± 5.91	7.52 ± 2.63	1.72 ± 0.83	38.93 ± 6.54	58.21 ± 8.55
补肾方低剂量组	10	45.21 ± 10.52	29.11 ± 6.90	7.62 ± 1.60	1.84 ± 0.72	22.31 ± 8.67	40.35 ± 5.44
补肾方中剂量组	10	41.02 ± 11.35	27.16 ± 6.35	7.49 ± 2.05	1.64 ± 0.47	40.81 ± 9.55	60.21 ± 7.53
补肾方高剂量组	10	35.80 ± 9.64	25.90 ± 7.64	7.20 ± 2.04	1.38 ± 0.37	54.72 ± 9.42	75.82 ± 10.44
F 值		3.291	3.256	2.460	3.779	9.519	5.317
P 值		0.019	0.020	0.059	0.010	0.000	0.009

管体积、血管表面积、血管体积分数、血管厚度均大于模型组（$P=0.000$，$P=0.000$，$P=0.000$，$P=0.000$；$P=0.009$，$P=0.003$，$P=0.002$，$P=0.001$；$P=0.000$，$P=0.001$，$P=0.001$，$P=0.000$；$P=0.007$，$P=0.015$，$P=0.011$，$P=0.005$）；补肾方低剂量组与模型组血管体积、血管表面积、血管体积分数、血管厚度比较，组间差异均无统计学意义（$P=0.051$，$P=0.052$，$P=0.082$，$P=0.064$）；补肾方低剂量组、补肾方中剂量组与健骨生丸组血管体积、血管表面积、血管体积分数、血管厚度比较，组间差异均无统计学意义（$P=0.057$，$P=0.063$，$P=0.051$，$P=0.052$；$P=1.000$，$P=0.222$，$P=1.000$，$P=0.813$）；补肾方高剂量组血管体积、血管表面积均大于健骨生丸组（$P=0.012$，$P=0.019$），2 组血管体积分数、血管厚度比较，组间差异均无统计学意义（$P=0.243$，$P=0.141$）；补肾方中剂量组和高剂量组血管体积、血管表面积、血管体积分数、血管厚度均大于低剂量组（$P=0.000$，$P=0.023$，$P=0.001$，$P=0.021$；$P=0.000$，$P=0.015$，$P=0.000$，$P=0.007$）；补肾方高剂量组血管体积、血管表面积、血管体积分数、血管厚度均大于中剂量组（$P=0.000$，$P=0.019$，$P=0.000$，$P=0.009$）。见表 3。

4　讨　论

临床和实验研究已经证实，长期使用或短期内大量使用激素均可能引起 ONFH[9-10]。目前，有关激素性 ONFH 发病的机理有脂肪代谢紊乱、血管内凝血、血管损伤等多种学说[11]，其中激素引起的血管损伤、血供中断被认为是激素导致 ONFH 的最后通路，而其他诸多发病机制很可能是应用激素后引起的中间过程或结果[12-13]。因此，采取有效的手段促进坏

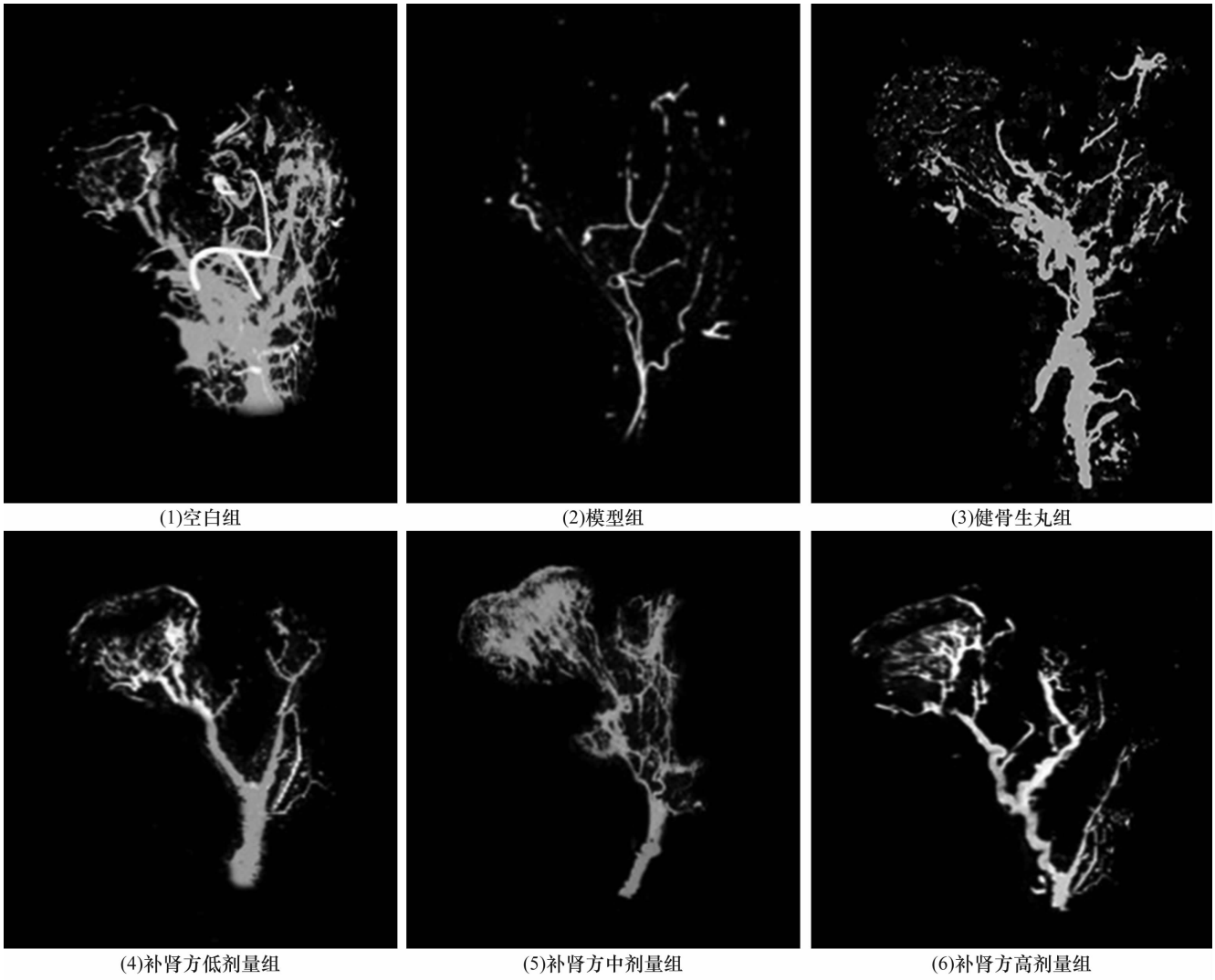

图 4　6 组大鼠股骨头血管 Micro－CT 检查结果

表 3　6 组大鼠股骨头血管形态检测结果　$\bar{x} \pm s$

组别	样本量（只）	血管体积（mm^3）	血管表面积（mm^2）	血管体积分数（%）	血管厚度（μm）
空白组	10	0.22 ± 0.05	10.01 ± 2.87	0.12 ± 0.03	62.93 ± 18.84
模型组	10	0.03 ± 0.01	4.20 ± 1.23	0.02 ± 0.01	22.73 ± 6.93
健骨生丸组	10	0.09 ± 0.02	5.93 ± 1.23	0.05 ± 0.02	33.63 ± 10.41
补肾方低剂量组	10	0.06 ± 0.04	5.13 ± 1.51	0.03 ± 0.01	28.53 ± 7.13
补肾方中剂量组	10	0.10 ± 0.02	6.15 ± 1.88	0.06 ± 0.02	35.16 ± 11.29
补肾方高剂量组	10	0.16 ± 0.05	8.45 ± 2.86	0.11 ± 0.03	51.80 ± 17.43
F 值		36.442	7.080	27.869	8.371
P 值		0.000	0.000	0.000	0.000

死区血管再生、重建血供和促进新骨生成，是治疗 ONFH 的关键[14]。

根据中医对 ONFH 病理发展过程中“骨痹—骨蚀—骨萎”的认识[15]，以及对其基本病机“肾虚骨病气衰血瘀”的理解，我们以补肾壮骨、活血化瘀为大法，组成补肾方，在临床中取得了一定的疗效[3]。该方中熟地黄、山药、山茱萸、杜仲、鹿角胶、菟丝子、狗脊能温补肝肾、益精血、破瘀血、续筋骨，肉桂补火助阳、散寒止痛，当归补血活血止痛，全方共奏补肾壮骨、活血通络之功。由于前期的动物实验已经证实补肾方具有促进激素性 ONFH 鸡和大鼠成骨和抑制破骨的作用[16-18]。故本实验中，我们采用大鼠激素性 ONFH 模型进一步探讨这一复方对激素性 ONFH 大鼠股骨头血管损伤的影响。和之前我们的系列研究报道[5-7]一样，这次仍然采取了在造模的同时就开始给药的方法，意在考察各组干预方法对激素性 ONFH

的防治作用。

为了比较补肾方的药效作用和特点,实验中我们选用了市售治疗 ONFH 的中成药健骨生丸作为阳性对照。健骨生丸由当归、三七、地龙、冰片、西红花、珍珠、冬虫夏草组成,具活血化瘀、通经活络、通血生骨功效,在临床用于瘀血阻络、筋骨失养所引起的ONFH的治疗,应用较为广泛。

实验中我们观察到补肾方能改善大鼠股骨头中空骨陷窝、脂肪细胞、骨小梁结构等组织病理形态异常改变。血管造影结合 Micro - CT 扫描技术是一项新的微血管定量方法,既能显示微血管的空间分布、走行、形态、联系和过渡关系,又能描述微血管表面形态以及血管内皮功能的状况,已被广泛应用于股骨头微循环状态和血管损伤的研究[19-20]。在实验中我们观察到激素性 ONFH 大鼠的股骨头内血管微结构模糊不清、血管较少不成网状,血管体积、血管表面积和血管厚度降低,而口服补肾方后,大鼠股骨头内血管微结构的清晰度和网状分布密度明显提高,血管体积、血管表面积和血管厚度也增加,说明了补肾方确实能促进激素性 ONFH 大鼠股骨血管修复,改善股骨头血液微循环状态,这也有助于股骨头内新骨生成。

VEGF 是重要的促血管生成调节因子,可特异性地作用于内皮细胞,与其受体 FLK1 结合,进而促进血管生成[6]。在激素性 ONFH 发病过程中,激素能抑制成骨和破骨细胞分泌 VEGF,使其在股骨头中的表达降低,进而抑制骨组织中的血管生成[21-24]。有学者发现 VEGF165 基因转染治疗能促进 ONFH 动物的血管新生[25]。本实验中,我们观察到激素性 ONFH 大鼠股骨头内 VEGF 和 FLK1 的蛋白表达降低,经补肾方治疗后,这些促血管新生因子的表达水平显著升高,提示了补肾方对激素性 ONFH 血管修复的作用可能和增加股骨头内 VEGF 和 FLK1 的蛋白表达有关。

已知大剂量应用激素能引起脂肪代谢紊乱和血液流变异常,造成 ONFH [11]。因此,本研究中我们还检测了激素性 ONFH 大鼠的血脂和血液流变指标。结果显示,补肾方能降低其血清 TG、TC、LDL 和 ApoB 含量,升高 HDL 和 ApoA1 含量,同时也能降低激素性 ONFH 大鼠全血黏度低切、中切值和血浆黏度。提示补肾方能在一定程度上改善激素性 ONFH 大鼠异常的血脂和血液流变指标。

本研究的结果提示,补肾方能促进激素性 ONFH 大鼠股骨血管修复,改善股骨头血液微循环状态,其作用可能与补肾方增加股骨头内 VEGF 和 FLK1 的蛋白表达有关,且补肾方中剂量的疗效与健骨生丸相当,补肾方高剂量的疗效优于健骨生丸。

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·通 知·

第 23 届全国中西医结合骨伤科学术年会征文通知

由中国中西医结合学会骨伤科分会主办,辽宁省中西医结合学会骨伤科分会、辽宁中医药大学附属医院承办,沈阳医学院附属中心医院协办的中国中西医结合学会骨伤科分会第 23 届全国中西医结合骨伤科学术年会将于 2016 年 9 月 16—18 日在辽宁省沈阳市召开。将邀请多位国内著名的骨伤科专家就骨伤疾病中西医结合特色诊治的最新国内、外进展进行专家论坛、专题讲座和疑难、典型病例讨论。现将会议征文要求通知如下。

征文内容 以中西医结合为特色的骨伤科疾病诊疗与防治。本次会议将涉及关节、创伤、脊柱、足踝、外固定、运动医学、骨质疏松、骨肿瘤、骨伤科基础研究、康复、护理等专业。涵盖创伤、关节、脊柱、足踝等骨伤疾病、软组织与运动医学损伤疾病、老年退行性骨伤疾病、骨与软组织肿瘤疾病的临床诊疗经验与诊疗技术规范研究,微创骨科外固定支架技术的临床应用及相关基础研究,骨伤科相关疾病临床和基础研究,骨伤科相关疾病药物治疗的临床及相关基础研究,康复与护理的相关临床及基础研究,与骨科相关的临床论著、基础研究英文版论文。

征文要求 所投论文应是未公开发表的。摘要宜 600 ~ 800 字,结构为目的、方法、结果、结论,并标注文章类别:关节、创伤、脊柱、足踝、外固定、运动医学、骨质疏松、骨肿瘤、骨伤科基础研究、护理、康复。论文请勿涉及保密内容,文责自负。本次会议只接收电子版稿件。请您将征文以 Word 格式发至投稿邮箱,邮件主题请注明“会议投稿”,务必注明工作单位、通讯地址、邮政编码及通讯作者的电子信箱、电话,以便及时通知您稿件录用情况。如您参加青年论坛(45 周岁以下),请务必在来稿中注明出生年月、电话及工作单位,并明确标注“青年论坛”。如您参加英文论坛(优秀论文推荐刊登至国内骨科唯一 SCI 收录期刊《Orthopaedic Surgery》),请注明“英文论坛”、电话及工作单位。

截稿日期 2016 年 8 月 15 日 24 时,以邮件发送时间为准。

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英文论坛联系方式 投稿地址:orthopaedicsurgery@126.com,邮件主题请注明“中西医结合骨伤科英文论文比赛投稿”;联系人:万瑜 13323350990,孙静 13821715917。

中国中西医结合学会骨伤科分会

2016 年 6 月 8 日