·基础研究·

液氮冷冻法诱导犬股骨头缺血性坏死模型 MRI 影像与病理观察

朱建龙，赵红昌

（浙江省杭州市萧山区第四人民医院，浙江 杭州 311225）

摘 要 **目的**：从 MRI 影像和病理表现 2 个方面研究液氮冷冻法诱导的犬股骨头缺血性坏死模型。**方法**：选取 12 只健康成年雄性 Beagle 犬，通过手术暴露其右侧股骨头后用一铁制漏斗套于股骨头，将 100～150 mL 液态氮倒入漏斗内，并维持冷冻 3 min，用 37 ℃温盐水将股骨头复温。左侧股骨作为对照。造模后即开始观察实验犬的一般情况，造模后 1 个月随机选取 6 只 Beagle 犬进行双侧股骨头 MRI 扫描及病理学检测，造模后 2 个月对剩余 6 只 Beagle 犬进行双侧股骨头 MRI 扫描和组织病理学检测。**结果**：①MRI 扫描。造模后 1 个月可见实验侧股骨头内于 T1W 上出现均质低信号，T2W 信号升高，STIR 信号升高更为明显，呈散在、点状分布于股骨头内；关节腔内可见少量关节积液；股骨头形态规则，边缘光滑，大小与对侧正常的股骨头一致。造模后 2 个月实验侧股骨头内于 T1W 上呈低信号，T2W 上呈略高信号，STIR 上呈略高信号；关节腔内积液；股骨头边缘不规则，变扁，变小；股骨头碎裂。②组织病理学观察。造模后 1 个月实验侧骨膜不完整，软骨部分脱落，软骨层变薄，软骨细胞排列紊乱；骨小梁稀疏变细，结构紊乱，有碎片出现，骨细胞陷窝空疏，骨细胞核固缩，部分血管栓塞；骨髓腔内造血组织明显减少，细胞数目及网状结构较正常稀疏，可见到大量脂肪细胞、多形核细胞、炎性细胞浸润，脂肪细胞体积增大，有的融合成泡状。造模后 2 个月实验侧关节面破坏严重，组织结构紊乱、破碎，关节软骨表层脱落、碎裂，软骨细胞排列紊乱，切线层变薄，同源软骨细胞数减少，软骨下潮线区变薄，钙化带与软骨下骨小梁不连续，呈“倒钟乳”状，软骨内可见到破骨细胞性骨吸收，形成髓腔。软骨下血管内可见血栓形成。骨基质中有大量的空骨陷窝，仅见少量骨细胞散在，细胞核固缩偏移。骨小梁稀疏、变细、不完整，出现微骨折，或骨小梁碎裂、紊乱，部分溶解呈片状。破骨细胞散在其间，骨髓组织坏死明显，有纤维组织增生修复，脂肪细胞肥大，也可见到其坏死、崩解后留下的空腔。骨髓内见出血区，造血细胞减少，大量脂肪细胞聚集，部分融合成大泡。**结论**：液氮冷冻法诱导的犬股骨头缺血性坏死模型符合股骨头坏死病变的一般规律，具有良好的重复性，是股骨头缺血性坏死的理想模型。

关键词 股骨头坏死 模型，动物 狗 磁共振成像 病理过程 液氮冷冻法

Observation on the MRI image and pathology image of the canine model with femoral head avascular necrosis induced by liquid nitrogen frozen

*ZHU Jian – long**，*ZHAO Hong – chang*. * *The Fourth People's Hospital of XiaoshanDistrict in Hangzhou City*，*Hangzhou* 311225，*Zhejiang*，*China*

ABSTRACT **Objective**：To study the canine model with femoral head avascular necrosis induced by liquid nitrogen frozen from the 2 aspects as MRI image and pathology image. **Methods**：Twelve healthy adult male beagle dogs were chosen for building models. Iiron funnel was set on the dog's right lateral femoral head exposed by operation，then 100 to 150 milliliter liquid nitrogen was infunded into the funnel and frozen state was maintained for 3 minutes，and next，the rewarming of femoral head was proceeded with 37℃ warm saline. The left lateral femoral head was served as the control. The general conditions of the experimental dogs were observed after modeling at once. One month after the modeling，6 beagle dogs were randomly chosen for MRI scanning and pathology testing in their bilateral femoral heads，and the same procedures were given to the other 6 dogs two months after the modeling. **Results**：①On the aspect of MRI scanning：one month after modeling，homogeneous low signals appeared on T1W images of the femoral head in experimental side，and signals enhanced on T2W images. Meanwhile，signals which scattered in a punctate pattern in the femoral head，enhanced more significantly on STIR images. A small amount of hydrops articuli was found within joint cavity. The shape of the femoral head was regular，while its edges were smooth and its size was consistent with the contralateral normal femoral head. Two months after modeling，low signals appeared on T1W images of the femoral head in experimental side，and slight hyperintensity signals appeared on T2W images and STIR images. There was hydrops articuli in joint cavity. The femoral head had irregular edges and appeared flat and smaller，or even broken into pieces. ②On the aspect of pathological observation：one month after modeling，the following phenomenon appeared in the femoral head in experimental side as incomplete periosteum，partly loss of cartilage，thinner cartilage layer，disordered arrangement of cartilage cells；thinner and sparse trabecular bone with disordered

structure and fragments; bone cells with loose and empty lacunes, karyopyknosis and partly embolismed blood vessel; significantly reduce of hematopoietic tissue in marrow cavity, less cells amounts and looser networks compared with the normal situations, appearance of infiltration with large amount of adipocyte, polymorphocyte and inflammatory cells, increased volume of adipocyte with some merged into bubbly shape. Two months after modeling, the following phenomenon appeared in the femoral head in experimental side as serious destruction of articular surface, disordered and shattered organization structure, abscission and fragmentation of articular cartilage surface, disordered arrangement of cartilage cells, thinner tangential layer, reduced amounts of isogenous chondrocytes, thinner tidal lines under the cartilage, discontinuation between calcified zone and subchondral trabecular bone which formed a shape as inverted stalactite, appearance of medullary cavity formed by osteoclastic bone resorption within cartilage; formation of thrombus in the subchondral vascular; large amount of empty bone lacunas and small amount of scattered bone cells in bone matrix, excursion and pyknosis of nucleus; sparse, thinner and incomplete bone trabecula with microfracture, or shattered and disordered bone trabecula with some merged into laminar shape; existence of scattered osteoclasts, prominent necrosis of myeloid tissue, appearance of fibroplasia repair, hypertrophy of fat cells with some of their left cavities after necrosis and disintegration; appearance of bleeding area in bone marrow, reduced amount of hematopoietic cells, aggregation of large amount of fat cells with some merged into bulla. **Conclusion**: The canine model with femoral head avascular necrosis induced by liquid nitrogen frozen, which is consistent with the general rule of femur head necrosis and is good in reproducibity, is a kind of ideal model for femoral head avascular necrosis.

Key words　Femur head necrosis; Models, animal; Dogs; Magnetic resonance imaging; Pathologic processes; liquid nitrogen frozen

目前早期股骨头缺血性坏死的动物模型众多,但是迄今仍然没有一种方法能完全模仿人股骨头缺血性坏死的病理过程。大多数模型只能反映股骨头缺血性坏死的早期病理变化;而不能复制出发生在人类缺血性坏死的股骨头上的软骨下骨折导致力学性质改变,股骨头塌陷,进入骨性关节炎阶段的模型。笔者应用液氮冷冻法造成 Beagle 犬股骨头缺血坏死,从 MRI 影像和病理表现 2 个方面进行了观察,现总结报告如下。

1　材料与仪器

1.1　实验动物　健康成年雄性 Beagle 犬 12 只,体质量(10 ±0.5)kg,由浙江中医药大学动物实验中心提供,实验动物合格证号:22 - 003657。

1.2　实验仪器　美国 GE 公司 Signa 1.5T MR 扫描仪;英国 Shandon 公司石蜡切片机。

2　方　法

2.1　造模方法　取实验 Beagle 犬右侧股骨进行造模,左侧作为对照。将 Beagle 犬用 3% 戊巴比妥钠静脉注射(1 mL · kg^{-1})麻醉后,固定于手术台上,右髋部剃毛,清洁皮肤,安尔碘消毒,按无菌操作原则进行手术。取髋关节后外侧切口,长约 5 cm,依次切开皮肤、筋膜,分离肌肉至关节囊。切开关节囊,暴露股骨头。股骨头周围用干纱布充填,以保护股骨头周围组织。用一铁制漏斗套于股骨头,将 100 ~ 150 mL 液态氮倒入漏斗内,并维持冷冻 3 min,用 37 ℃温盐水将股骨头复温,逐层缝合切口,无菌敷料覆盖。术中及术后静脉滴注 5% 葡萄糖盐水 500 mL + 庆大霉素 24 万 U,术后第 2 天和第 3 天改用青霉素 160 万 U 肌肉注射。术后圈内饲养,任其自由活动。

2.2　效果观察　造模后即开始观察实验犬的一般情况,造模后 1 个月随机选取 6 只 Beagle 犬进行 MRI 扫描及病理学检测,造模后 2 个月对剩余 6 只进行 MRI 扫描和组织病理学检测。

2.2.1　MRI 扫描　选用冠状面 T1W、T2W、STIR 及横断面 T1W、T2W 扫描,层厚 3 mm,层距 0.5 mm,矩阵为 256 ×192。观察股骨头轮廓、骨质密度及骨小梁变化,以及是否有囊性变或硬化、股骨头皮质下新月状透亮影及内部裂隙样透亮线形成。

2.2.2　组织病理学检测　取双侧股骨头,沿股骨头凹冠状面剖成 2 半,置于 10% 甲醛溶液中固定 48 h,0.27 mol · L^{-1}EDTA - 2Na(PH = 7.4,温度 4 ℃)脱钙 3 个月,每周更换 1 次脱钙液。待股骨头脱钙完全后,将所有标本放至脱水机中应用梯度乙醇脱水(75% →85% →95% →无水乙醇)。然后经二甲苯透明后石蜡包埋,切片,HE 染色。观察股骨头的组织及细胞结构改变情况。

3　结　果

3.1　一般情况　造模后前 3 d 实验犬精神萎靡,摄食减少,倦卧少动,右侧后足不愿活动,行走时明显跛行,并且右后足被自己咬破。3 d 后动物神态正常,皮毛顺滑,摄食、睡眠、二便及活动量均正常,右侧后足明显跛行,跑跳时经常滑倒。

3.2　股骨头外观　造模 1 个月后，实验侧股骨头变扁平，轮廓光滑，软骨面完整，无缺损，剖面见关节软骨厚度基本一致，软骨下骨小梁可见。造模 2 个月后，实验侧股骨头扁平，呈蘑菇状。其中 1 只 Beagle 犬股骨头可见骨质明显增生，表面粗糙不平，软骨有缺损，负重区有面积不等的软骨塌陷，颜色淡红，有散在分布的白色斑，剖面见关节软骨厚薄不均，凹凸不平，关节软骨负重区有塌陷(图 1)。

3.3　MRI 扫描　造模后 1 个月可见实验侧股骨头内于 T1W 上出现均质低信号，T2W 信号升高，STIR 信号升高更为明显，呈散在、点状分布于股骨头内，关节腔内可见少量关节积液；股骨头形态规则，边缘光滑，大小与对侧正常的股骨头一致[图 2(1)]。2 个月后实验侧股骨头内 T1W 低信号，T2W 略高信号，STIR 略高信号；关节腔内积液，股骨头边缘不规则，变扁，变小，股骨头碎裂[图 2(2)]。

3.4　组织病理学观察　①造模后 1 个月，正常股骨头骨小梁结构完整、粗大、密集，排列整齐，骨小梁中的骨细胞清晰可见，空骨陷窝少见；软骨层结构完整，软骨细胞排列整齐；骨髓中造血细胞丰富，骨髓腔内造血组织丰富，脂肪组织相对较少，脂肪细胞形态正常，小血管正常，结构完整，血管内皮细胞丰富[图 3(1)]。实验侧骨膜不完整，软骨部分脱落，软骨层变薄，软骨细胞排列紊乱；骨小梁稀疏变细，结构紊乱，有碎片出现，骨细胞陷窝空疏，骨细胞核固缩，部分血管栓塞；骨髓腔内造血组织明显减少，细胞数目及网状结构较正常稀疏，可见到大量脂肪细胞、多形核细胞、炎性细胞浸润，脂肪细胞体积增大，有的融合成泡状[图 3(2)]。②造模后 2 个月实验侧关节面破坏严重，组织结构紊乱、破碎，关节软骨表层脱落、碎裂，软骨细胞排列紊乱，切线层变薄，同源软骨细胞数减少，软骨下潮线区变薄，钙化带与软骨下骨小梁不连续，呈“倒钟乳”状，软骨内可见到破骨细胞性骨吸收，形成髓腔，软骨下血管内可见血栓形成；骨基质中有大量的空骨陷窝，仅见少量骨细胞散在，细胞核固缩偏移；骨小梁稀疏、变细、不完整，出现微骨折，或骨小梁碎裂、紊乱，部分溶解呈片状，破骨细胞散在其间；骨髓组织坏死明显，有纤维组织增生修复，脂肪细胞肥大，也可见到其坏死、崩解后留下的空腔，骨髓内见出血区，造血细胞减少，大量脂肪细胞聚集，部分融合成大泡[图 3(3)]。

4　讨　论

理想的股骨头缺血性坏死动物模型除了要求与人类的股骨头缺血性坏死有相似性和可比性外，还应具备以下几个特征：①符合人类股骨头坏死病变的病理过程；②模型有良好的可重复性；③动物的解剖和

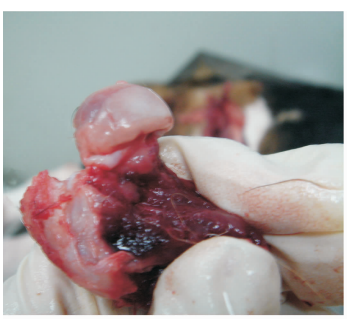
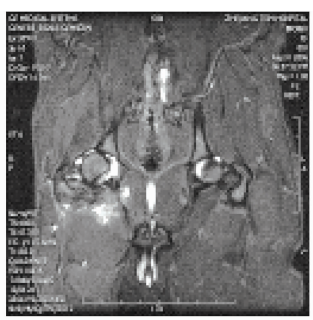
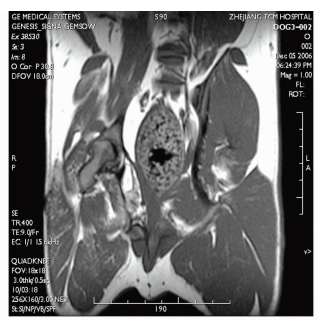


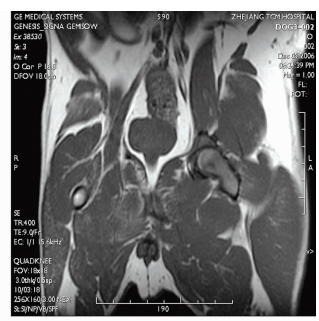
图1　造模后2个月Beagle犬股骨头外观



(1) 实验侧造模后1个月

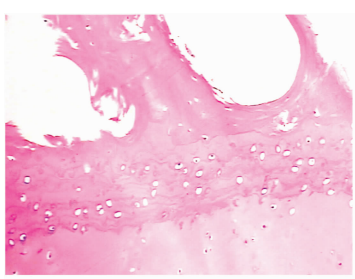


(2) 实验侧造模后2个月



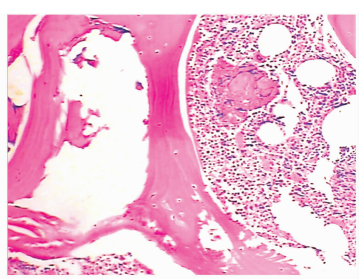
(3) 对照侧

图2　Beagle犬股骨头MRI扫描图像



(1) 造模后1个月对照侧

(2) 造模后1个月实验侧



(3) 造模后2个月实验侧

图3　Beagle 犬股骨头组织病理学观察(HE 染色　×400)

(1)软骨层结构完整　(2)骨膜不完整，软骨部分脱落骨小梁稀疏变细，结构紊乱

(3)骨基质中有大量的空骨陷窝，仅见少量散在的骨细胞

生理特点尽可能与人类相似;④经济可行。灵长类动物解剖及生理特点与人类近似,是制造股骨头缺血性坏死模型最理想的动物。但是由于灵长类动物存在驯养困难、费用昂贵、实验周期较长等问题,影响了它们的使用。而犬的解剖和生理特点与人类相似、温顺听话、费用适中、实验周期短,因此本实验选用 Beagle 犬作为实验动物。

制作股骨头缺血性坏死模型的常用方法是激素诱导法。Miyanishi 等[1]对不同类型糖皮质激素致股骨头坏死的作用进行比较,结果发现醋酸甲基泼尼松龙具有相对较强的致股骨头坏死作用,激素型股骨头坏死模型虽可出现不同程度的坏死但与人类的情况仍存在很多不同之处。此外,还有多种股骨头缺血性坏死模型制作方法,如股骨头颈截断及结扎股骨头供血血管的创伤模型,酒精灌胃的酒精性股骨头缺血性坏死模型,减压病股骨头缺血性坏死模型,内毒素股骨头缺血性坏死模型,液氮冷冻股骨头缺血性坏死模型及股骨头缺损股骨头缺血性坏死模型等。

非创伤性股骨头缺血性坏死的发病机制至今尚未完全明了,但所有股骨头缺血性坏死的病理变化是一致的,即骨细胞坏死和随后的修复反应[2]。Hanzeur 等[3]提出的早期股骨头缺血性坏死判断标准为:①至少有部分骨小梁坏死,存在 50% 以上的空骨陷窝;②异常骨髓出现在坏死骨小梁周围;③无局部感染和肿瘤病灶存在的证据。Takaoka 等[4]首次建立了液氮冷冻股骨头坏死模型,之后许多研究者复制、改良其方法。如杨述华等[5]利用液氮冷冻成功地建立了兔股骨头坏死模型。顾晓峰等[6]手术暴露犬股骨头并用液氮冷冻,同时破坏股骨头基底部血管和软组织,术后 11 周承重区软骨下骨见明显囊性改变,承重区塌陷。王江泳等[7]采用液氮棉球冷冻成年新西兰大白兔双侧股骨头负重区建立股骨头坏死模型。液氮冷冻建立股骨头缺血性坏死模型的机理可能是液氮冷冻可引起血管痉挛、血管内皮细胞受损、血管内凝血、血管通透性增加,继而发生出血和复温后的缺血再灌注损伤,最终导致股骨头坏死。而且液氮冷冻还不同于化学方法造成的坏死,不会残留化学物质。另外,该方法造模所需时间短,坏死成功率高,动物死亡率低,可重复性好[8]。通过液氮冷冻建立的模型,由于冷冻立即使股骨头呈完全性坏死,而且逐步开始修复反应,是一个坏死程度和坏死时间可以控制的标准化的动物模型,适合于治疗研究。

本实验采用了液氮冷冻方法成功制作了犬股骨头缺血性坏死模型。它成功地表现了股骨头的坏死和修复过程。造模后 1 个月 MRI 可见实验侧股骨头内于 T1W 上出现均质低信号,T2W 信号升高,STIR 信号升高更为明显,呈散在、点状分布于股骨头内,关节腔内可见少量关节积液,股骨头形态规则,边缘光滑,大小与对侧正常的股骨头一致。病理切片示骨膜不完整,软骨部分脱落,软骨层变薄,软骨细胞排列紊乱;骨小梁稀疏变细,结构紊乱,有碎片出现;骨细胞陷窝空疏,骨细胞核固缩;骨髓腔内造血组织明显减少,细胞数目及网状结构较正常稀疏,可见到大量脂肪细胞、多形核细胞、炎性细胞浸润等,脂肪细胞体积增大,有的融合成泡状。

因此,笔者认为液氮冷冻法诱导的犬股骨头缺血性坏死模型符合股骨头坏死病变的一般规律,具有良好的重复性,是股骨头缺血性坏死的理想模型。

5　参考文献

[1] Miyanishi K, Yamamoto T, Irisa T, el al. Effects of different corticosteroids on the development of osteonecrosis in rabbits[J]. Rheumatology (Oxford), 2005, 44(3): 332 – 336.

[2] Jones LC, Hungerford DS. The pathogenesis of osteonecrosis [J]. Rheumatology (Oxford), 2005, 44(3): 332 – 336.

[3] Hauzeur JPH, Pasteels JL. Bone biopsy as diagnostic criteria for aseptic necrosis of the femoral head[M]//Schoutens A, Arlet J, Gardeniers JWM, et al. Bone circulation and avascularization in normal and pathological conditions. New york: Plenum Pless, 1993: 277.

[4] Takaoka K, Yoshioka T, Hosoya T, et al. The repair process in experimentally induced avascular necrosis of the femoral head in dogs[J]. Arch Orthop Trauma Surg, 1981, 99(2): 109 – 115.

[5] 杨述华,杨操,许伟华,等. 液氮冷冻建立兔股骨头缺血性坏死模型[J]. 中华矫形外科杂志,2001,8(1):48.

[6] Gu XF, Li FF, Fan WM, el al. The experimental research of setting up the subsiding model of avascular necrosis of femoral head[J]. Acta Univ Med Nanjing, 2006, 26(12): 1224 – 1226.

[7] 王江泳,王保芝,崔慧先,等. 改良液氮冷冻法制备家兔股骨头坏死模型的形态学研究[J]. 河北医科大学学报,2008,29(1):5 – 7.

[8] 彭吾训,龚跃昆,李世和,等. 液氮冷冻建立犬股骨头缺损坏死模型的实验研究[J]. 中国康复,2005,20(2):76 – 77.

(2010-03-17 收稿　2010-05-24 修回)