李勇进,章晓云,曾浩. 骨质疏松症病证结合动物模型的研究进展[J]. 中国实验动物学报, 2024, 32(6): 772-785.
LI Y J, ZHANG X Y, ZENG H. Progress in the establishment and evaluation of osteoporosis disease and syndrome combination animal models[J]. Acta Lab Anim Sci Sin, 2024, 32(6): 772-785.
Doi:10.3969/j.issn.1005-4847.2024.06.010

骨质疏松症病证结合动物模型的研究进展

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【摘要】 随着老龄化的加剧,骨质疏松症(osteoporosis,OP)的发病率逐年升高,已成为全球公共卫生问题之一。其主要特征为骨量降低及骨微结构破坏,临床上常表现为疼痛、驼背、身高降低,甚至出现骨折。积极进行与 OP 相关的科学研究显得尤为重要,动物模型的构建已经成为参与医药研究的关键手段之一,尤其是在探索新的治疗方法和药物开发过程。其中,融合了中医药特色的"病证结合"动物模型在我国中医药现代化研究中占据着不可替代的地位。本文从目前病证结合 OP 动物模型的现状出发,总结模型构建及评价方法,以期为病证结合 OP 动物模型的研究提供一定的思路及参考。

【关键词】 骨质疏松症;病证结合;动物模型;研究进展
【中图分类号】Q95-33 【文献标志码】A 【文章编号】1005-4847(2024)06-0772-14

Progress in the establishment and evaluation of osteoporosis disease and syndrome combination animal models

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【**Abstract**】 With increased population aging, the incidence of osteoporosis (OP) increases year by year, and has become a key global public health problem. Its main characteristics are reduced bone mass and damage to bone microstructure, often clinically manifesting as pain, hunched-back posture, height reduction, and even bone fractures. Therefore, it is particularly important to actively carry out scientific research related to OP, and the construction of animal models has become one of the key drivers of medical research, especially for exploring new treatments and in drug development. Animal models that are a "combination of disease and syndrome", integrating the characteristics of Chinese medicine, occupy an irreplaceable position in research aiming to modernize traditional Chinese medicines. On the basis of

[基金项目]国家自然科学基金(82360937),广西自然科学基金面上项目(2023GXNSFAA026075),广西中医药适宜技术开发与推广项目(GZSY22-36),广西中医药多学科交叉创新资助课题(GZKJ2304),广西高校壮医毒病研究重点实验室(桂教科研[2022]10号),黄有荣桂派中医大师培养项目(桂中医药科教发[2022]6号),广西中医药大学青年创新研究团队项目(2021TD001)。
Funded by National Natural Science Foundation of China (82360937), Guangxi Natural Science Foundation Project (2023GXNSFAA026075), Guangxi Traditional Chinese Medicine Appropriate Technology Development and Promotion Project (GZSY22-36), Guangxi Traditional Chinese Medicine Multidisciplinary Interdisciplinary Innovation Funding Project (GZKJ2304), Key Laboratory of Zhuang Medicine Poison Research in Guangxi Universities (Gui Jiao Research [2022] No. 10), Huang Yourong Gui School TCM Master Training Project (Gui TCM Science and Education Fa [2022] No. 6), Youth Innovation Research Team Project of Guangxi University of Chinese Medicine (2021TD001).
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the current research status of disease-syndrome combination OP animal models, this paper summarizes model construction and evaluation method to provide certain ideas and references for research using these specialized OP animal models.

【Keywords】 osteoporosis; combination of disease and syndrome; animal models; research progress

Conflicts of Interest: The authors declare no conflict of interest.

骨质疏松症(osteoporosis, OP)是一种全身性代谢性骨骼疾病,其主要病理特征在于骨质丢失造成的低骨量及骨微结构的恶化[1]。OP 在临床上无明显症状[2],但患者全身骨脆性增加,容易发生骨折,给患者个人及社会带来沉重的经济负担。随着我国老龄化加剧,OP 发病率逐年上升,已成为我国主要的公共健康问题之一,被称为“二十一世纪的无声流行病”[3]。流行病学调查结果显示,50 岁以上人群 OP 患病率为 19.2%,其中女性为 32.1%、男性为 6.9%,而 65 岁以上人群 OP 患病率为 32.0%,其中女性为 51.6%、男性为 10.7%[4]。OP 长期发展及其带来的并发症如骨骼畸形、骨折等,对人们的正常生活产生了严重影响。

目前,西医对 OP 的研究多偏向于病理方面,临床多以地舒单抗、降钙素、雌激素等药物进行对症治疗,但存在诸多禁忌症和毒副作用。中医药在治疗 OP 过程中具有价廉、易获得、毒副作用小等优势。西医对 OP 所采用的研究方法相对单一,多是从病理或临床单方面进行,而中医药研究更倾向于将 OP 的西医病理机制与中医证候辨证相结合,即病证结合,已成为医学科学发展的重要手段。通过构建 OP 病证结合动物模型,形成客观、科学的模型评价体系,对中医药防治 OP 及中医药现代化进程的研究具有重大意义。本文通过中西医临床两个角度对疾病的认识进行总结,归纳出 OP 病证结合动物模型的构建方法和存在的问题,总结提炼不同证候的量化指标,为如何构建符合临床发病机理的病证结合动物模型及评价体系提供科学客观的依据。

1　OP 的发病机制

1.1　OP 西医发病的病因病理

虽然 OP 发病机制尚未明确,但破骨细胞活性提高,成骨细胞凋亡,进而影响人体骨代谢异常是目前学者们公认的主流发病机制之一[5]。正常成年人中,骨骼自身存在代谢周期,被称为骨代谢[6]。骨代谢的平衡是指破骨细胞对骨吸收速度与成骨细胞对骨形成速度大致相同,但当机体因衰老、激素水平、疾病、各种机械损伤及药物等影响时,出现成骨转化能力减弱或骨吸收能力增强的情况,则会伴随出现骨量丢失的现象,最终引发 OP。如氧化应激条件下大量活性氧堆积,从而引发成骨细胞脂质过氧化损伤而凋亡;再如贯穿机体骨发育过程中的铁离子出现代谢失衡,最终出现铁过载,导致成骨细胞铁死亡发生,而破骨细胞活性增强,也已成为 OP 的重要发病机制之一[7](见图 1)。

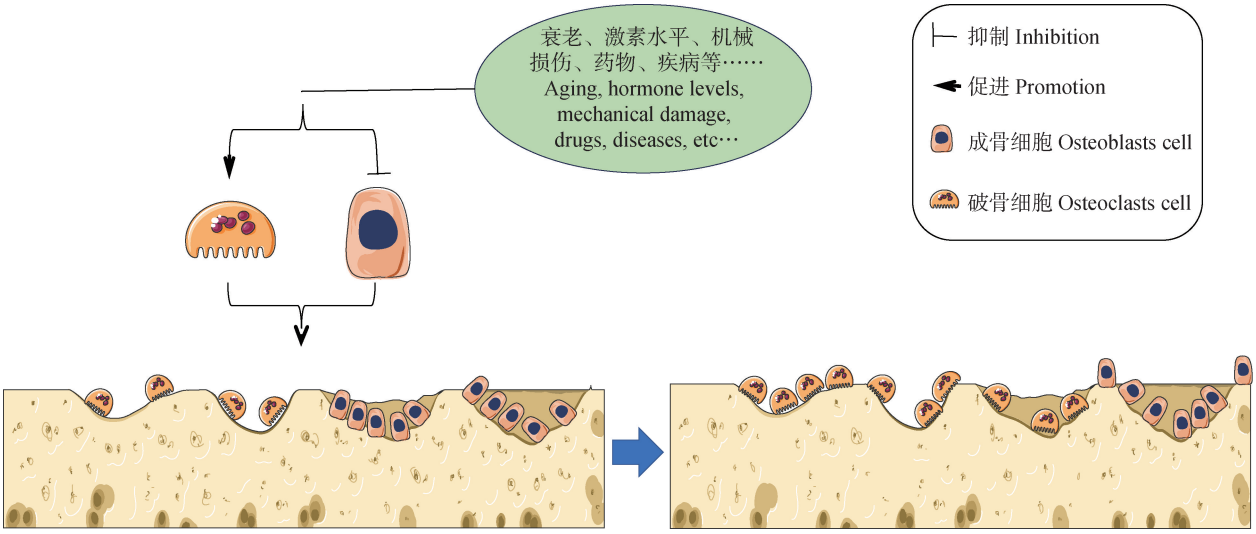

图 1　骨质疏松发生机制

Figure 1　Pathogenesis of osteoporosis

1.2 OP 中医发病的病因病机

中医并无 OP 病名,但按其症状及发病机理,可将其归属于“骨痹”“骨痿”“骨枯”等范畴。《素问·痿论篇》曰“肾主身之骨髓……肾气热,则腰脊不举,骨枯而髓减,发为骨痿”,认为肾主骨、生髓,肾精亏虚是 OP 发病的主要诱因。《黄帝内经》认为肾禀赋于先天之精,主骨并且依赖于后天之精的充养。脾为后天之本,气血生化之源,若后天水谷精微补充不足,或恣食肥甘厚味、嗜酒、偏食等伤及脾胃,则骨无以滋养发生骨痿[8]。林崙镌等[9]研究表明脾肾虚弱使肌肉充养不利,骨髓失养导致肌少症,从而诱发 OP,其认为脾、肾与 OP 病因病机之间存在紧密联系。刘金勇等[10]研究表明龟鹿补骨方可通过滋补肝肾之阴从而有效治疗绝经后骨质疏松症,证实肝肾阴虚,精血化源不足,则骨枯而痿的病因病机。

2 OP 的诊断标准

2.1 西医诊断标准

参照《原发性骨质疏松症诊疗指南(2022)》[4]及国内外相关研究[11-17],临床症状、影像学检查、实验室检查、骨密度检测是诊断 OP 的 4 个主要关键点,其中骨密度检测作为临床诊断最重要的手段之一,是诊断 OP 的“金标准”,应将其列为核心指标,影像学及实验室检查可作为相对客观的评价手段,将其列为主要指标,临床症状缺乏特异性,将其列为次要指标,详见表 1。

2.2 中医诊断标准

参考《绝经后骨质疏松症(骨痿)中医药诊疗指南(2019 年版)》及相关研究[18-21]发现,目前临床上最主要的证型为肾虚证型。不同证型各自所特有的临床表现应将其列为核心指标,而彼此之间存在的相似临床表现作为诊断 OP 的次要指标,见表 2。

3 动物模型的研究现状

目前,OP 基础实验中所需动物模型主要采用病理造模、病因造模以及病证结合造模 3 种方式,造模动物多为单一哺乳动物,如大鼠[22]、小鼠[23]、犬[24]等。由于 OP 的临床症状、体征并无明显的特异性,因此大部分学者多以病因造模为主。动物模型成功与否也多采用是否符合 OP 临床症状或病理机制的单一因素进行评价,如绝经后骨质疏松症的动物模型,其评价方式只是通过检测骨密度及雌二醇水平。即便造模后动物的外在表现符合 OP 的临床症状或病理机制,但是生理生化指标与人体差异性很大,无法判断模型是否对应 OP 中医证候类型。

由于无法采用中医评价体系判断单一因素的

表 1 OP 的西医诊断标准

Table 1 Western medical diagnostic criteria for osteoporosis

诊断要素 Elements of diagnosis	诊断方法 Methods of diagnosis	指标性 Indexability
骨密度测试[11-13] BMD testing[11-13]	①DXA/DEXA:T 评分等于或小于-2.5;②QCT:绝对 BMD 值小于 80 mg/cm^3;③TBS:TBS 值小于或等于 1.200。 ①DXA/DEXA. T score equal to or less than -2.5. ②QCT. Absolute BMD value less than 80 mg/cm^3. ③TBS. TBS is less than or equal to 1.200.	核心指标 Core indicators
影像学[14-16] Imaging[14-16]	①X 线检查:椎体内结构呈纵行条纹,周围骨皮质变薄,椎体变扁,上下缘内凹,椎间隙增宽;②CT:椎体中央或整个区域骨松质密度减低,CT 值有时低达-90 HU,有时椎体松质骨骨小梁呈粗点状、蜂窝状改变、骨皮质可见普遍变薄以及椎体周边骨质增生;③MRI:椎间盘变薄、呈低信号其内可见不规则斑点状高信号区,椎间隙距离增加。 ①X-ray examination. Longitudinal stripes in the internal structure of the vertebra, thinning of the peripheral bone cortex, flattening of the vertebral body, concave of the upper and lower edges, and widening of the intervertebral space. ②CT. Density of cancellous bone in the center or the entire area of the vertebral body is reduced, the CT value is sometimes as low as -90 HU, sometimes the trabecular bone of the vertebral cancellous bone is coarsely punctate, honeycomb changes, the bone cortex can be seen as general thinning, and the bone hyperplasia around the vertebral body. ③MRI. Intervertebral disc is thinned and low-signal, with irregular spot-like hyper-signal areas visible and intervertebral space distances increased.	主要指标 Main indicators
实验室检查[17] Laboratory examination[17]	①血钙浓度降低;②骨钙素降低;③血清碱性磷酸酶降低;④血清抗酒石酸酸性磷酸酶升高等。 ①Blood calcium concentration decreased. ②Decreased osteocalcin. ③Serum alkaline phosphatase decreased. ④Serum anti-tartrate acid phosphatase increased, etc.	
临床症状[4] Clinical signs[4]	①腰背部疼痛或全身性骨痛;②脊柱出现畸形形变;③身高降低或体态出现驼背畸形;④髋部或椎体发生脆性骨折。 ①Low back pain or generalized bone pain. ②Deformity of the spine occurred. ③Decreased height or humpback deformity. ④Fragility fractures of the hip or vertebral body.	次要指标 Secondary indicators

表 2　OP 主要证型的中医诊断标准

Table 2　Main diagnostic criteria of osteoporosis in traditional Chinese medicine

证型 Syndrome type	特有表现(核心指标) Characteristic presentation(Core indicators)	相似表现(次要指标) Similar presentation(Secondary indicators)
肾阳虚证[18] Kidney-Yang deficiency[18]	①小便频数;②舌淡苔白、脉沉细弦 ①Frequency of urine. ②Pale tongue with whitish coatin, deep pulse and thin string	①腰背冷痛;②酸软乏力;③驼背弯腰 ①Cold pain in the back and waist. ② Acidity, weakness, and fatigue. ③Hunchback
肝肾阴虚证[19] Liver-kidney Yin deficiency syndrome[19]	①眩晕耳鸣;②五心烦热;③舌红少津、少苔、脉沉细数 ①Vertigo and tinnitus. ②Dysphoria in chestpalms-soles. ③Dry redness of tongue, lacking tongue coating, pulse sinking, fine and fast	①腰膝酸软无力,抽筋;②驼背弯腰;③形体消瘦 ① Lumbar and knee soreness and weakness, cramps. ②Hunchback. ③Physical emaciation
脾肾阳虚证[19] Spleen and kidney Yang deficiency[19]	①小便不利或频多;②大便久泄不止或五更泄泻;③舌淡胖,苔白滑,脉沉细弱或沉弦迟 ①Poor or frequent urination. ②chronic diarrhea or Wugeng diarrhea. ③ Pale and plump tongue, white and smooth tongue coating, dim and weak pulse or deep and taut pulse	①酸软乏力,甚则驼背弯腰;②活动受限 ①Acidity, weakness, and even stooping. ②Activity limitations
肾虚血瘀证[20] Kidney deficiency and blood stasis[20]	①步履艰难;②舌质淡紫,脉细涩 ①Trudge. ②Tongue light purple, fine and astringent pulse	①腰脊刺痛;②腰膝酸软;③下肢萎弱 ①Lumbar spine tingling pain. ②Lumbar debility. ③Lower limb weakness
脾胃虚弱证[21] Spleen and stomach deficiency[21]	①面色萎黄或浮肿或消瘦;②纳少脘胀、便溏;③舌淡苔白,脉细弱无力 ①Yellowish or swollen or emaciated complexion. ②Lack of appetite, bloating, diarrhea. ③ Pale tongue with whitish coating, thin and delicate pulse	①腰背酸软、疼痛;②肌肉萎缩;③筋骨羸弱 ①Low back soreness and pain. ②Muscle atrophy. ③Weakness of muscles and bones
血瘀气滞证[19] Syndrome of blood stasis and Qi stagnation[19]	①痛有定处,拒按;②筋肉挛缩;③舌质紫暗,有瘀点或瘀斑,脉涩或弦 ① Pain has a fixed point, refuse to press. ② Muscle contracture. ③ Tongue purple dark, petechiae or ecchymosis, pulse astringency or stringency	①腰背及周身疼痛;②驼背弯腰 ①Pain in the back and body. ②Hunchback

OP 模型是否与中医证型对应,因此,OP 造模方式需要采取相较于单因素造模更合理的造模方式。将“外在”的证候和“内在”的病理指标结合进行多因素造模,才能构建同时符合西医病理机制与中医证型的动物模型,即 OP 病证结合造模。评价 OP 病证结合模型是否建立成功,不能简单地将造模动物的临床表现与 OP 西医临床症状或病理机制相对应,应当加入 OP 中医临床证候的评价要素,观察造模动物的症状表现与 OP 西医临床症状的符合程度(见表 3)。

4　OP 病证结合动物模型评价体系的探讨

评价病证结合动物模型应当以 OP 病理模型为基础,同时观察动物证候表现,如体重、毛发、爪甲、体态及二便等情况,当病理与证候表现二者完全符合或基本符合 OP 的临床诊断标准及不同证型的证候表现时,则造模成功。评价体系的关键是如何将不同证型模型的证候表现进行标准化。评价指标的收集与归纳也是不可或缺的一环,特别是中医证候的收集,由于中医临床所依赖的四诊合参在动物模型上并不能及时有效地收集到合理且客观的 OP 证候表现,因此需要借助现代技术的帮助,如体温计测量动物模型肛温以收集体温改变[35]、心电图测量心跳以收集动物模型脉搏[36]、通过舌象仪采集动物模型舌象[37]等。不同证型 OP 的证候特征彼此之间有共用性与特异性,在收集归纳特异性证候表现中,需要选择能与西医病理机制相互联系的证候表现。

4.1　OP 不同证型模型共用量化指标

无论何种证型的 OP 均可通过西医理论中 OP 的发病机制及病理表现一一对应阐释,而中医学认为病理表现与“证候表现”的关系密切,西医的病理生化指标相较于临床表现,拥有更高的敏感度,能及时捕捉到不同证型造模的细微变化。因此将西医病理生化指标作为造模评价体系的基础,并与中医证候相交叉,找到不同证型 OP 的共有病理生化指标,对造模评价体系标准的建立具有非常重要的意义(见表 4)。研究表明,骨代谢异常是导致 OP 发生的主要机制,骨保护素[38]、碱性磷酸酶[39]、骨钙素[40]等含量会因骨形成异常而发生病理性减少;当骨吸收发生异常时,核因子-κB 受体活化因子和核因子-κB 受体活化因子配体[41]、抗酒石酸酸性磷

表 3 不同 OP 动物模型造模的特点

Table 3 Characteristics of different OP animal models

造模类型 Modeling type	造模方法 Modeling method	动物 Animal	特点 Aharacteristic	符合症状 Conforming to symptoms	
				西医 Western medicine	中医 Traditional Chinese medical
病理造模 Pathological modeling	坐骨神经切断法[25] Sciaticnerve amputation method[25]	Wistar/SD 大鼠、C57BL/6 小鼠、新西兰长耳白兔等 Wistar/SD rats, C57BL/6 mice, New Zealand long eared white rabbits, etc	均采用了手术方式进行造模。简便、高效、造模所需时间短,但会增加术后感染及死亡的风险。 All models were made using surgical methods. It is simple, efficient, and requires a short time for modeling, but it increases the risk of postoperative infection and death.	血钙浓度降低、骨钙素降低、骨小梁稀疏、变细。 Blood calcium concentration decreases, osteocalcin decreases, and bone trabeculae become sparse and thinner.	体重下降、反应迟钝、动作迟缓。 Weight loss, slow reaction, and delayed movement.
	卵巢切除去势法[26] Ovariectomy and castration method[26]	SD 大鼠、Wistar 大鼠、C57BL/6 小鼠等 SD rats, Wistar rats, C57BL/6 mice, etc			
	糖皮质激素法[27] Glucocorticoid method[27]	SD 大鼠、C57BL/6 小鼠、新西兰大白兔、斑马鱼(AB/TU 品系)等 SD rats, C57BL/6 mice, New Zealand white rabbits, zebrafish (AB/TU strain), etc	不会对造模动物造成机械性损伤,同时更符合人体激素分泌水平。但是所需要时间长,药物本身存在干扰。 It will not cause mechanical damage to model animals and is more in line with human hormone secretion levels. But it takes a long time and the drug itself interferes.		
	维甲酸灌胃法[28] Retinoic acid gavage method[28]	SD 大鼠 SD rats	操作简单、建模时间短、骨质疏松症状典型,但停用维甲酸后骨质疏松状态逐渐恢复。 Operation is simple, the modeling time is short, and the symptoms of osteoporosis are typical. However, after discontinuing retinoic acid, the osteoporosis status gradually recovers.		
	乙醇法[29] Ethanol method[29]	SD 大鼠、Wistar 大鼠 SD rats, Wistar rats	建模材料简单、方法简便易行,是探讨乙醇对骨代谢影响的重要模型,应用较多。但由于乙醇用量无法具体控制,因此无法准确断定造模成功所需要的具体时间。 Modeling materials are simple, and the methods are simple and feasible. It is an important model for exploring the impact of ethanol on bone metabolism and has many applications. Since the amount of ethanol used cannot be controlled, it is not possible to determine exactly how long it will take for the mold to be successful.		
	链脲佐菌素法[30] Streptozotocin method[30]	SD 大鼠 SD rats	建模方法成熟,建模周期较长,但模型的发病过程及临床表现与人类糖尿病相类似,多用于研究糖尿病性 OP。 Modeling method is mature and the modeling period is long, but the pathogenesis and clinical manifestations of the model are similar to those of human diabetes, and it is mostly used to study OP in diabetes.		

续表 3

造模类型 Modeling type	造模方法 Modeling method	动物 Animal	特点 Aharacteristic	符合症状 Conforming to symptoms	
				西医 Western medicine	中医 Traditional Chinese medical
病因造模 Pathogenic modeling	注射 RANKL(核因子-κB 受体活化因子配体)[31] Injection of RANKL (nuclear factor κB receptor activator ligand)[31]	SD 大鼠 SD rats	可模拟高周转率骨病模型,并用于表征 RANKL/RANK/OPG 通路在多种骨病中的潜在作用。 It can simulate a high turnover bone disease model and be used to characterize the potential role of the RANKL/RANK/OPG pathway in various bone diseases.	血钙浓度降低、骨钙素降低、骨小梁稀疏、变细。部分模型出现血清抗酒石酸酸性磷酸酶升高、脆性骨折。 Blood calcium concentration decreases, osteocalcin decreases, and bone trabeculae become sparse and thinner. Some models exhibit elevated serum tartrate resistant acid phosphatase and fragile fractures.	动作迟缓、肛温升高、小便黄赤、大便干结、毛发干枯。 Slow movement, elevated rectal temperature, yellow urine, dry stools, and dry hair.
	皮下注射注射链脲佐菌素[32] Subcutaneous injection of streptozotocin[32]	SD 大鼠 SD rats	造模方法成熟,造模周期较长,但模型的发病过程及临床表现与人类发病相类似即在造模动物体内选择性破坏胰岛 β 细胞,使其诱发血糖升高,增加骨质流失,符合 OP 病因。 Modeling methods are mature and the modeling cycle is long, but the pathogenesis and clinical manifestations of the model are similar to those of humans, that is, selective destruction of pancreatic islets in modeling animals β cells can induce elevated blood sugar and increase bone loss, which is consistent with the etiology of OP.		
	维甲酸灌胃 + 氢化可的松臀部肌注[33] Intragastric administration of retinoic acid + intramuscular injection of hydrocortisone into the buttocks[33]	SD 大鼠 SD rats	取材简便,同时减少了机械损伤对于造模因素的干扰,有效模拟了 OP 的肾阳虚型。 Easy to obtain materials while reducing the interference of mechanical damage on modeling factors. Effectively simulated the kidney Yang deficiency type of OP.		共同症状:体重下降、反应迟钝、毛色不光。① 肾阳虚:精神萎靡、拱背蜷曲;② 肾阴虚:大便干结、小便黄赤;③脾肾阳虚:大便稀溏,甚者如水状。 Common symptoms: weight loss, slow reaction, and uneven hair color. ① Kidney Yang deficiency: mental exhaustion, arched back curling. ② Kidney Yin Deficiency: Dry and dry stools, yellow urine. ③ Spleen and Kidney Yang Deficiency: Loose and loose stools, sometimes resembling water.
病证结合造模 Combination of disease and syndrome modeling	肌注地塞米松注射液[27] Intramuscular dexamethasone injection[27]	SD 大鼠 SD rats	造模方式简单、高效,药物取材方便且对造模因素外的干扰很小,有效模拟了 OP 的肾阴虚型。 Modeling method is simple and efficient, with convenient drug extraction and minimal interference from modeling factors. Effectively simulated the kidney Yin deficiency type of OP.	骨小梁稀疏变细、骨钙素降低、血清抗酒石酸酸性磷酸酶升高、发生脆性骨折等。 Sparse and thinning of bone trabeculae, decreased osteocalcin, increased serum tartaric acid phosphatase, and occurrence of brittle fractures.	
	注射氢化可的松 + 番泻叶灌胃[34] Injecting hydrocortisone + senna leaves by gavage[34]	SD 大鼠 SD rats	造模药物便宜易获得、造模简单易操作、干扰因素小,有效模拟了 OP 的脾肾阳虚型。 Modeling drugs are cheap and easy to obtain, and modeling is simple and easy to operate. Interference factor is small. Effectively simulated the spleen and kidney Yang deficiency type of OP.		

酸酶[42]含量会显著上升。因此,上述与骨代谢相关指标水平改变可作为 OP 动物模型的共用量化评价指标。另外,血钙[43]、血磷[44]及肾功中肌酐[45]、血清胱抑素 C 水平以及尿素氮[46]等也会因为骨代谢异常而发生改变,这些指标也可作为不同证型 OP 动物模型的共用量化评价指标。PDGF-BB[47]蛋白在临床研究中较为不常见,但可以通过调控骨骼中微血管形状(尤其是 H 形血管)影响骨骼的生长发育、修复、重塑和代谢等过程。因此,微血管形状及其数量也可作为共用量化指标之一。

中医理论认为“肝肾同源,精血同源”,肝肾两脏之间的关系密切[48]。若肝血不足,一方面使肾精亏虚,则髓枯骨空;另一方面血虚肢体筋骨失养、肢体活动不利等,因肝血虚所引起的骨痿是目前临床 OP 发病的主要病机之一[49]。《素问·阴阳应象大论篇》云“肾生骨髓,髓生肝”,即肝血不断濡养肾精使肾精化生有源,肾精使肝血充沛,两者之间是精血互化的关系[50]。因此,OP 的 6 种证型都会在不同时期出现肝肾亏虚的表现,而肝肾亏虚的临床表现则可作为 OP 中医不同证型模型的共用量化指标。目前肝肾亏虚型 OP 的临床表现主要有骨节或腰脊酸痛软弱、头晕、耳鸣、步履艰难、持重困难等[51]。因此在建立 OP 模型时,若 OP 动物模型在满足西医共用量化指标的同时,也满足以上中医共用量化指标表现在动物身上的体征,如毛发干枯、饮水增多、易怒、四肢萎软等,则可认为成功构建 OP 病证结合动物模型。

4.2 OP 不同证型模型特异性量化指标

OP 的 6 种证型虽然都存在肝肾亏虚表现,但是每个证型也都存在各自特有的临床表现。因此,有必要收集不同证型所特有且与西医病理机制相互联系的临床表现,作为该证型的特异性量化指标(见表 4)。

4.2.1 肾阳虚

肾阳虚是指肾阳气亏虚,脏腑温煦功能下降。临床表现可见腰膝酸软、畏寒肢冷、精神不振、舌淡胖苔白、脉弱无力[62]等。动物肾阳虚的造模方式普遍选择水应激、劳倦过度等外部环境因素干预构建体重下降、反应迟钝、毛色不光、精神萎靡、拱背蜷曲等相应表现[63]。但这种方式不仅造模过程冗杂,同时也无法提供特异性量化指标以及评价造模是否成功[64]。肾阳虚模型可以将肾阳虚所特有的表现,如畏寒肢冷、精神倦怠、反应迟钝等表现与甲状腺功能减退所诱发的体温降低、血压下降、精神倦

表 4 OP 不同证型模型的共用/特异性量化指标

Table 4 Quantitative indicators of OP specificity for different syndrome types

证型 Syndrome type	共用量化指标 Shared quantitative indicators	特异性量化指标 Specificity quantification
肾阳虚 Kidney-Yang deficiency	骨保护素[38]、碱性磷酸酶[39]、骨钙素[40]、核因子-κB 受体活化因子和核因子-κB 受体活化因子配体[41]、抗酒石酸酸性磷酸酶[42]、血钙[43]、血磷[44]、肌酐[45]、血清胱抑素 C 和尿素氮[46]、微血管形状及其数量[47] Osteoprotein[38], Alkaline phosphatase[39], Osteocalcin[40], Nuclear factor-κB receptor activator and nuclear factor-κB receptor activator factor ligands[41], Resistant to tartaric acid phosphatase[42], Blood calcium[43], Blood phosphorus[44], Creatinine[45], Serum cystatin C and urea nitrogen[46], Microvascular shape and its number[47]	总三碘甲状腺原氨酸[52]下降、总甲状腺素[52]下降、体温[53]下降 Total-triiodothyronine[52] decreases, Total-thyroxine[52] decreases, Body temperature[53] decreases
肝肾阴虚 Liver-kidney Yin deficiency syndrome		C-反应蛋白[54]升高、平均动脉压[54]升高、水通道蛋白 1[55]升高、水通道蛋白 3[55]升高 C-reactive protein[54] rises, Mean arterial pressure[54] rises, Aquaporin 1[55] rises, Aquaporin 3[55] rises
脾肾阳虚 Spleen and kidney Yang deficiency		尿 D-木糖代谢率[56]下降 Decreased urine D-xylose metabolic rate[56]
肾虚血瘀 Kidney deficiency and blood stasis		内皮素[57]升高、血小板聚集率[58]升高、血浆粘稠度[58]升高 Elevated endothelin[57], Elevated platelet aggregation rate[58], Increased plasma viscosity[58]
脾胃虚弱 Spleen and stomach deficiency		胃泌素[59]降低、胃动素[59]降低 Gastrin[59] decreases, Motilin[59] decreases
血瘀气滞 Syndrome of blood stasis and Qi stagnation		痛阈值[60]改变、血清纤维蛋白原[61]升高、造模动物骨关节前后照片[60]改变 Pain threshold[60] changes, Serum fibrinogen[61] rises, Pre- and Post-joint photographs of modeled animals[60] changes

怠、反应迟钝[65]等表现相联系,通过摘除甲状腺的方式进行造模,可能体现该模型的特异性量化指标,即总三碘甲状腺原氨酸(tatol-triiodothyronine, TT3)和总甲状腺素(total-thyroxine, TT4)[52]、体温[53]。由于 TT3、TT4 的血清含量会在甲状腺功能减退时发生明显下降[66];同时,发生甲状腺功能减退时,体温也会相应下降[67]。

4.2.2　肝肾阴虚

肝肾阴虚既是 OP 最常见的证型之一,也是 OP 主要病机[68]。有许多中医临床特异性较强的表现,如手足心热、大便干结、舌红少苔、脉细数等[69],与交感神经兴奋以及体液分泌减少诱发的心跳加快[54]、大便干结[70]等表现十分相似。因此,在建造肝肾阴虚模型时,需要找到与交感神经及体液分泌有关的特异性量化指标。以及当交感神经兴奋时,C-反应蛋白(C-reactive protein, CRP)与平均动脉压(mean arterial pressure, MAP)显著升高。与体液分泌有关的特异性指标有水通道蛋白 1(Aquaporin 1, AQP1)、水通道蛋白 3(Aquaporin 3, AQP3)在血清中含量增加时,会减少肠道黏液的分泌[55]。因此,可以采用电刺激/牵拉交感神经、灌注左甲状腺素钠等方式构建 OP 肝肾阴虚模型,在构建过程中定期测量造模动物 CRP、MAP 和血清中 AQP1 及 APQ3 的含量作为肝肾阴虚模型的特异性量化指标。

4.2.3　脾肾阳虚

脾肾阳虚是脾阳虚与肾阳虚的结合,以脾阳虚为主,损及肾阳引起[71]。完谷不化是特异性最强的临床表现,与西医病理机制中的脾功能关系密切,脾功能中最具代表性的量化指标尿 D-木糖代谢率与脾功能呈正相关[56]。因此在构建脾肾阳虚模型时可以先将造模动物通过番泻叶或大黄制剂灌胃,以抑制造模动物脾功能,造模前中后三期分别监测造模动物的尿 D-木糖代谢率。若尿 D-木糖代谢率相较于造模前期及中期出现下降趋势,则 OP 脾肾阳虚模型建立成功。

4.2.4　肾虚血瘀

该证型在 OP 各证型中最为少见,造模方式也少有人提及。多数 OP 肾虚血瘀模型的构建都是通过先构建肾虚证型再叠加血瘀证型。舌色淡紫与脉细涩是肾虚血瘀各类表现中特异性最强的表现。舌色淡紫与脉细涩在西医可以解释为血管收缩过度,血液循环受阻。因此,建立肾虚血瘀模型时,可以通过结扎左冠状动脉前降支以及配合惊吓的方式建立肾虚血瘀模型。左冠状动脉前降支血流减缓后,内皮素(endothelin, ET)升高[57]、血小板聚集率和血浆粘稠度[58]升高。若 ET 含量升高、血浆粘稠度和血小板聚集率升高,则 OP 肾虚血瘀模型造模成功。

4.2.5　脾胃虚弱

脾胃虚弱模型的构建与西医糖尿病性 OP 模型构建方式相同,即使用糖皮质激素诱导动物发生 OP[72]。该证型与脾肾阳虚证型有诸多相似之处,二者都是以脾虚为主要证型,但脾胃虚弱证相较于脾肾阳虚证更加偏向于统摄失权、运化无力等脾气虚弱的临床表现,与西医病理机制中消化不良所诱发的四肢乏力、腹部胀痛、厌食等症状相似[73]。胃泌素(gastrin, GAS)、胃动素(motilin, MTL)水平会因为消化不良的影响而显著降低[59],可作为脾胃虚弱证型的特异性量化指标。因此,通过饮食不节以及抑制有关 GAS、MTL 分泌的蛋白表达率来构造模型,若在造模后 MTL、GAS 水平发生显著下降,则证明脾胃虚弱模型构建成功。

4.2.6　血瘀气滞

《医林改错》中:“元气既虚,必不能达于血管,血管无气,必停留而瘀。”瘀血是 OP 最常见的病理因素,主要由于肝失条达导致气机阻滞、血行不畅,气血津液不能正常输布而导致筋骨失养,气血阻滞于脉络之间。最特异性的临床表现与瘀血有关,如关节刺痛、痛有定处、关节肿胀变形等[60]。这一系列特异性表现可通过痛阈值改变[61]、血清纤维蛋白原(fipinogen, FIB)含量改变[61]以及拍摄造模动物骨关节前后照片对比而反映。由于关节的肿胀变形及疼痛,造模动物痛阈值会降低[60];同时,瘀血的产生会使造模动物 FIB 明显升高[74]。因此,在建立 OP 血瘀气滞模型时,可以对造模动物采用机械损伤增加粘连的方式构建血瘀气滞模型。若造模后痛阈值降低、FIB 显著升高及对比其造模前后关节发生肿胀变形,则表明血瘀气滞模型构建成功。

5　病证结合动物模型的具体场景和意义

动物模型的核心作用便是通过动物模型的构建提供研究者可施加干预的平台,其意义是通过特殊干预手段模拟人体病理改变,以获得客观的数据资料。6 种不同的 OP 证型的具体场景(见表 5)。

表 5 OP 不同证型模型的具体场景与建模方式

Table 5 Specific scenarios and modeling methods of different syndrome models of OP

证型 Syndrome type	具体场景 Specific scene	构建方式 Construction mode
肾阳虚[75] Kidney-Yang deficiency[75]	研究老年性 OP 时可使用此类模型 This model can be used to study senile OP	通过灌胃药物[76]等方法抑制甲状腺功能造成甲状腺激素水平降低 Thyroid function was inhibited by intragastric drugs[76] and the level of thyroid hormone was reduced
肝肾阴虚[77] Liver-kidney Yin deficiency syndrome[77]	研究糖尿病性 OP、PMOP 时可使用此类模型 This model can be used in the study of diabetic OP and PMOP	通过机械刺激交感神经并结合减少饮水[78] By mechanical stimulation of the sympathetic nerve combined with reduced water intake[78]
脾肾阳虚[79] Spleen and kidney Yang deficiency[79]	研究老年性 OP、肌少性 OP 时可使用此类模型 This model can be used in the study of senile OP and sarcopenia OP	通过大黄或番泻叶制剂进行多次低剂量灌胃,以大便不成形为标准[80] Multiple low-dose intragastric administration was performed with rhubarb or senna preparations, with fecal imformation as the standard[80]
肾虚血瘀[81] Kidney deficiency and blood stasis[81]	研究老年性 OP、PMOP 中后期时可使用此类模型 This model can be used to study the middle and late stages of senile OP and PMOP	通过结扎左冠状动脉减缓血流速度并结合恐吓[82] Ligation of the left coronary artery slows down the flow rate and is combined with intimidation [82]
脾胃虚弱[83] Spleen and stomach deficiency[83]	研究糖尿病性 OP、肌少性 OP 可使用此类模型 This model can be used to study diabetic OP and sarcopenia OP	通过禁止动物进食或不规律喂食的方式[84] By prohibiting animals from eating or feeding them irregularly[84]
血瘀气滞[85] Syndrome of blood stasis and Qi stagnation[85]	研究各类 OP 中后期时及其出现骨折并发症时可使用此类模型 This model can be used in the study of various OP in the middle and late stages and in the occurrence of fracture complications	通过束缚等方式增加动物机械损伤并结合药物诱发二次炎症形成粘连[86] Mechanical damage of animals was increased by means of restraint and combined with drugs to induce secondary inflammation to form adhesion[86]

6 总结和展望

目前对于 OP 动物实验的开展,主要是以大鼠、小鼠、兔、狗等动物为主,针对不同实验需求,基本都采用单一因素造模方法。开展病证结合研究可以遵循中医辨证论治的实质,也体现了西医对 OP 的发病机制和病理表现。严格控制动物模型不同证候之间的关键量化因素,可以有效模拟人体 OP 症状,采用西医病理学检查手段,可以有效研究出 OP 发病机制中的特异性指标为中西医结合及其中医药的现代化提供了一种新的路径。过去评价造模成功的方式往往单纯采用西方医学的评价体系再叠加中医“证侯”的评价体系,缺乏规范性和标准性,建立合理的模型评价体系于 OP 病证结合动物模型而言是目前急需解决的问题。

本研究通过总结能联系中医的“证”与西医的“病”的特异性量化指标,以期建立客观合理评价 OP 病证结合动物模型的体系。在此基础上,发展 OP 病证结合动物模型的应用,为构建 OP 病证结合动物模型提供新的评价思路及构建方式。

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[收稿日期]　2023-10-08