·综述·

老年性骨质疏松症动物模型及其发病机制的研究进展

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摘要:老年性骨质疏松症(SOP)是生物衰老在骨骼方面的一种特殊表现,对老年人的健康和生活质量造成了严重的威胁。随着人口老龄化的加快,如何防治 SOP 及其引发的骨折不仅成为了医学界急需解决的问题,也是一个社会化问题。建立合适的 SOP 动物模型能更好地指导其发病机理的探究,并为新药的研发提供帮助。本文主要总结了近几年来应用较多的 3 种 SOP 动物模型,包括 SAMP6 快速老化 SOP 动物模型、D-半乳糖致衰老性 SOP 动物模型、自然衰老性 SOP 动物模型,并对以上动物模型各自的发病机制进行综述。

关键词:老年性骨质疏松;动物模型;机制

Research progress of animal models and the pathogenic mechanism of senile osteoporosis

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Abstract: The special appearance of biological ageing in skeletal system is senile osteoporosis (SOP), which impairs seriously the healthy state and life quality of the elderly. With the acceleration of population aging, how to prevent and treat SOP and SOP-induced osteoporotic fracture is a problem to be solved urgently in medical field, and is also a social problem. The development of appropriate animal models for SOP can benefit to explore the pathogenesis of SOP and to help the research and development of novel drugs. This review summarizes 3 SOP animal models, including the senescence-accelerated mouse strain P6, the D-galactose-induced aging animal model, and the naturally aged animal model, which are more often used in recent years, and reviews the respective pathogenesis of each animal model.

Key words: Senile osteoporosis; Animal model; Mechanism

老年性骨质疏松症(SOP)又称为Ⅱ型骨质疏松症,表现为“低转换”型骨质疏松,主要是由于老年人骨髓基质细胞向成骨细胞方向分化受抑制,成骨细胞分裂增殖缓慢,骨形成因子合成代谢受阻、活性衰退致骨形成期延长,骨形成率降低,同时破骨细胞对骨的吸收增强,使骨重建处于负平衡,骨量丢失[1]。它以骨量减少、骨组织微结构破坏为主要特征,女性一般发生在绝经后 20 年以上,男性多发生于 70 岁以上。SOP 的严重并发症为骨质疏松性骨折。Datta[2]指出,65 岁以上老年人患骨质疏松性骨折的死亡率很高,且仅有不足 50% 的人能恢复到骨折前的功能水平。根据不同病因建立合适的老年性骨质疏松症动物模型,模拟 SOP 临床症状和病理学变化,对于深入认识 SOP 的病理机制、新药的研发和有效治疗方案的制定具有重要意义。

1 快速老化性骨质疏松动物模型

SAM(Senescence-accelerated mouse)是由日本学者开发的系列快速老化动物模型,分为快速老化 P 品系和正常老化 R 品系。其中,SAMP6 是已建立的快速老化骨质疏松动物模型。它的成骨细胞数目少、活力低,在 4~5 月时骨量达到峰值,然后随着年龄的增长其骨小梁体积减小,骨量和骨强度逐渐降低并伴有成骨细胞形成下降,且有自发性骨折倾向,是目前唯一被证实可随年龄增长而出现脆性骨折的动物模型[3]。

SAMP6 模型早期就表现出了骨量下降和骨重建减少,这与骨髓间充质干细胞(mesenchymal

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stromal cells, MSCs)向成骨细胞的分化不足有关。MSCs 广泛存在于骨髓和松质骨中,对于维持骨吸收和骨形成的动态平衡具有重要作用。SOP 患者发病的一个主要原因就是老年人成骨细胞分化增殖能力降低而脂肪细胞分化增殖能力增加,这就造成了骨髓腔内脂肪的积累[4],脂肪的堆积又会威胁成骨细胞的生存并抑制其发挥作用[5]。对从 SAMP6 小鼠长骨中分离的 MSCs 的研究,同样发现了与临床上 SOP 患者相一致的情况[6]。研究表明[7],在骨代谢过程中 TGF-β1 信号通路会通过诱导 MSCs 的迁移,抑制其向脂肪细胞的分化而向成骨细胞分化来调控骨重建。基因芯片通路分析发现[8],相比于同龄正常老化 SAMR1 小鼠,SAMP6 的 TGF-β1 信号通路中有四个基因 MAPKl、INHBA、DCN、GDF7 存在显著性差异,这四个基因可能在 MSCs 的成骨与成脂分化平衡中发挥重要作用。

此外,白细胞介素-11(IL-11)也参与 MSCs 向成骨细胞方向的分化调控,它的过表达可预防由增龄引起的皮质骨丢失[9]。体外实验表明[10],IL-11 通过上调 STAT3 表达而提高骨形态发生蛋白(bone morphogenetic protein, BMP)特异性转录因子 Smad1 的转录活性来活化 BMP-2 信号通路,进而诱导 MSCs 向成骨细胞分化并抑制其成脂分化。与同龄正常老化 SAMR1 小鼠相比,SAMP6 小鼠股骨 BMP-2 表达明显降低[11]。Ueda 等[12]将 SAMP6 小鼠的骨髓细胞直接注入 C57BL/6 小鼠骨髓腔内,1 个月后,发现后者也显示出了骨质疏松的特征,IL-11 的基因表达与 SAMP6 体内的表达结果一致,均显著下降。给予重组人 IL-11 后,成骨细胞的形成得以恢复,脂肪形成作用减弱。

2 D-半乳糖致衰老性骨质疏松动物模型

D-半乳糖致雄性鼠骨质疏松症是一个较为实用的研究老年男性骨质疏松的动物模型。大量食用 D-半乳糖后,睾丸功能减退,雄激素水平降低,这与衰老致性腺功能退化相似[13]。此外,体内会产生过多的自由基而破坏胶原蛋白的形成,造成矿物质流失,加速雄性鼠的衰老,导致其骨量减少,骨质丢失[14]。Hung 等[15]选取 12 周龄雄性 Wistar 大鼠作为研究对象,实验组动物进行腹腔注射 150 mg/(kg·d)的 D-半乳糖。8w 后,发现该模型大鼠骨质流失,骨小梁数量减少,股骨体积减小,骨孔隙密度增加,且模型组大鼠的几种重要矿物质如钙、镁、锰、磷含量相比于同龄正常大鼠均有显著降低[16],证明长期服用 D-半乳糖可导致老年性骨质疏松症。

D-半乳糖可转换为晚期糖基化终产物(AGEs),它不能被进一步代谢而在组织中积累[17]。如图 1 所示,AGEs 的堆积会增加成骨细胞中蛋白水解酶 caspase-3、caspase-8、caspase-9 的活性,从而诱导成骨细胞的凋亡,导致骨形成减少。AGEs 还能增加成骨细胞生成核因子 κB 受体活化因子配体(RANKL),刺激破骨细胞的骨吸收因子即 IL-6 的形成,而 RANKL 能促进破骨细胞的分化和活化并抑制其凋亡,IL-6 也会增加破骨细胞活性导致骨吸收加快[18,19]。此外,通过基因芯片技术和分子生物学实验研究[20],发现 AGEs 通过激活 ROS-P38 介导的信号通路促进趋化因子 Ccl2, Ccl3, Ccl4 等分泌来抑制 MSCs 的迁移和向成骨细胞的分化。

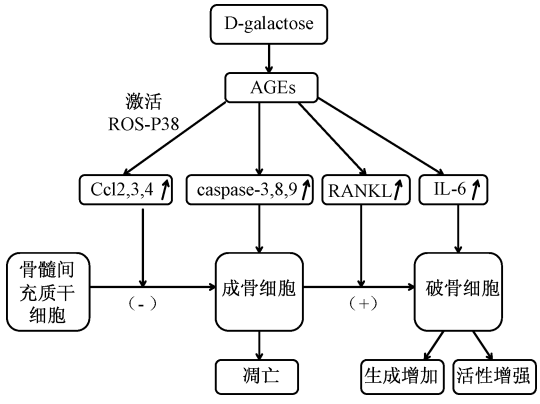

图 1 D-半乳糖的晚期糖基化终产物(AGEs)对骨代谢的调控通路

Fig. 1 Regulation pathway of advanced glycosylation end products (AGEs) from D-galactose on bone metabolism

3 自然衰老性骨质疏松动物模型

研究衰老导致的骨质疏松,理想的动物模型是采用自然衰老的老年动物,从模型的形成机理来讲,它与 SOP 最为接近。

现代医学研究表明,SOP 是在衰老过程中发生的一种骨组织的生理性退变,其退变的程度受多种因素的影响,而端粒酶就在其中扮演着重要角色[21]。Brennan 等[22]选取 15 月龄雄性 C57BL/6J 小鼠作为自然衰老性骨质疏松动物模型,同时,对同种 10 月龄小鼠构建端粒破坏小鼠模型($Terc^{-/-}$)。结果显示,两种模型均出现骨小梁数量减少,体积减小,且皮质骨变薄,孔隙度增加,这可能与端粒酶介导成骨细胞的分化形成有关。Wang 等[23]通过对 $Terc^{-/-}$ 小鼠模型 MSCs 的体外培养研究发现,端粒

功能障碍会通过刺激 P53/P21 通路来诱导 MSCs 的衰老与凋亡,同时下调 MSCs 中成骨细胞转录因子 Runx2 的表达而抑制 MSCs 向成骨细胞的转化、分化,使骨量减少[24]。

近年来,作为一种新型氧化应激标志物,晚期蛋白氧化产物(AOPPs)在许多老年相关性疾病中的重要作用引起了广泛关注。AOPPs 可诱发 SOP 的发生[25]。Zeng 等[26]选取 18 月龄雄性 SD 大鼠作为研究对象,发现 AOPPs 会加速老年鼠骨质恶化,这主要是由于 AOPPs 与晚期糖基化终产物受体(RAGE)结合后,在 NADPH 氧化酶的催化下促使活性氧(ROS)生成,且增加 RAGE 的表达,进而通过抑制 Wnt,PI3K、ERK 通路抑制成骨细胞增殖[27]。经 NADPH 氧化酶抑制剂—夹竹桃麻素处理后,大鼠骨退化则明显减轻[28]。

除上述机理外,临床研究还发现[29],随着年龄的增长雌激素水平逐渐缺失也是导致 SOP 发生的一个重要原因,尤其是对老年女性。雌激素缺失会激活 T 细胞释放与骨吸收相关的细胞因子 TNF-α、IL-6、RANKL 等[30]。最新研究结果显示[31,32],在雌激素缺乏引起的 SOP 中,TNF-α 能够通过下调一个关键 miRNA-miR-21 的表达来抑制 MSCs 向成骨细胞的分化,而 miR-21 已被证实可抑制 Spry1 的表达调控骨形成过程。

4 总结与展望

理想的 SOP 动物模型对于抗老年性骨质疏松症药物的研究具有重要意义。作为新药研究的模型应尽可能在发病机制、病变过程、病理改变等方面与临床骨质疏松症相似。复制老年性骨质疏松动物模型一般选择 18 ~ 24 月龄的动物,它与临床 SOP 最为接近,但是,老年动物饲养过程中死亡率较高,日常饲养、管理费用较高。SAMP6 模型可以加快动物的衰老速度,且模型特点与人老年性骨质疏松症病理表现非常相似,是研究 SOP 较为实用的动物模型。D-半乳糖致雄性鼠骨质疏松动物模型是研究男性 SOP 的一个重要工具,相比于其他 SOP 动物模型简便易行、价格低廉。但是,值得关注的是 D-半乳糖对雌性小鼠不会引起骨量丢失而不能导致骨质疏松。随着研究的深入和新技术的发展,更适合的动物模型将不断出现,SOP 的临床治疗方法也将不断得到改进。

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