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博来霉素诱导的肺纤维化动物模型研究进展

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【摘要】 肺纤维化(pulmonary fibrosis,PF)是一种进行性、间质纤维化性肺疾病,其特征是肺实质的持续瘢痕形成,患者生存质量降低、预后不良。PF 发病机制不明,缺乏有效治疗药物,动物模型是探讨疾病发病机制、寻求有效治疗药物的主要工具。多种因素可诱导纤维化形成,根据已知病因(如粉尘、辐射、细菌或病毒等)可不同程度诱导 PF 模型,其中,博来霉素(bleomycin,BLM)诱导的模型具有重复性强、纤维化病理与临床类似等优势而被广泛应用,其诱导方式主要有气管内滴注、气管内雾化、尾静脉注射、腹腔注射和经鼻吸入,根据诱导频次分为单次和多次诱导。本文基于相关文献,总结了 BLM 不同诱导频次、诱导方式建立的 PF 模型的特点,为其应用提供依据。

【关键词】 肺纤维化;动物模型;博来霉素;造模方法
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Progress in animal models of bleomycin-induced pulmonary fibrosis

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【Abstract】 Pulmonary fibrosis (PF) is a progressive, interstitial fibrotic lung disease characterized by persistent scar formation in the lung parenchyma, and a reduced quality of life and poor prognosis for patients. The pathogenesis of PF is unknown and there is a lack of effective therapeutic agents; however, animal models are currently the main tool used to explore the pathogenesis of the disease and to find effective therapeutic agents. PF can be induced by various factors and to different degrees according to known etiologies. Among these, bleomycin-induced models are widely used because of their reproducibility and the similarity between the fibrosis pathology and clinical conditions. The main induction method include intratracheal drip, intratracheal nebulization, tail vein injection, intraperitoneal injection, and transnasal inhalation, and these can be classified into single and multiple doses, according to the frequency of induction. Based on the relevant literature, the current review summarizes the characteristics of the bleomycin-induced PF model using different induction frequencies and method, to provide a basis for the application of this model.

【Keywords】 pulmonary fibrosis; animal model; bleomycin; modeling method
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肺纤维化(pulmonary fibrosis, PF)是一种慢性、进行性、不可逆的间质性肺疾病,是多种类型弥漫性肺间质疾病(diffuse interstitial lung disease, DILD)的共同结局[1-2]。PF 病因复杂,发病机制尚不明确,研究认为其与环境暴露、吸烟、感染、遗传易感性、衰老等因素有关[3]。PF 目前缺乏有效的治疗药物,经美国食品药品监督管理局(Food and Drug Administration, FDA)批准的吡非尼酮、尼达尼布虽然能在一定程度上改善临床症状,减缓肺功能下降,但存在一定副作用及药物耐受性问题[4-5],肺移植是 PF 唯一治愈方法,仅可作为少数患者的治疗选择[6]。因此,加强对 PF 的机制研究和新药物研发是目前医学界迫切需要解决的问题。

动物模型是进行疾病基础研究的一个主要工具,稳定可靠、可重复、符合人类病理特征的动物模型是研究疾病发病机制及有效治疗的重要基础,虽然目前没有一种动物模型能概括人类 PF 的全部特征,但仍能部分再现 PF 的病理表现[7],这对于评价药物疗效、探究药物作用机制具有重要意义。用于诱导 PF 的动物模型包括鼠、猫[8]、狗[9]、马[10]、兔[11]、羊[12]等,其中,鼠类具有造模周期短、实验效率高、个体差异小、价格便宜、可重复性强等优点,是最常用的造模动物[13],多种诱导因素均可引起肺纤维化的发生,如博来霉素(bleomycin, BLM)、石棉[14]、二氧化硅[15]、异硫氰酸荧光素(fluorescein isothiocyanate, FITC)[16]、辐射[17]、百草枯[18]等,不同诱导方式各有优缺点,见表 1。其中,BLM 诱导的肺纤维化模型操作简单、可重复性强、与人类疾病的典型特征相似,是目前应用最广泛的 PF 模型[19]。本文总结了常用的 BLM 诱导 PF 模型,并分析这些动物模型的应用及特点,为肺纤维化动物模型的应用提供参考。

1 博来霉素诱导肺纤维化的历史渊源

BLM 是一种氨基糖肽类抗生素,1966 年,日本学者首先从轮枝链霉菌的培养液中分离得到,FDA 于 1973 年批准了 BLM 的临床使用,具有良好的抗肿瘤活性,是一线抗肿瘤药物[20-22]。然而,随着 BLM 使用的增加,不久后便发现由于肺部产生的博来霉素水解酶(BLM 的主要灭活剂)水平较低,易发生破坏性的毒副作用,造成肺纤维化或严重的间质性肺病[23-24]。有赖于这种毒副作用,1974 年 Adamson 等[25]首次采用腹腔多次注射 BLM(每只 0.5 mg),每周 2 次,连续 4 周,发现 BLM 可引起小鼠肺间质和肺泡持续性损伤,这种改变与人肺纤维化相似,从而为研究 PF 提供了一种合适的动物模型。1978 年 Snider 等[26]通过一次性气管内滴注 BLM(0.5 U/100 g)成功建立了仓鼠 PF 动物模型。自此,BLM 诱导的 PF 模型被广泛应用于肺纤维化的实验研究。

2 博来霉素诱导肺纤维化模型的现状

大量实验证实 BLM 单次或多次给药均可诱导 PF 模型,诱导方式有气管内滴注、气管内雾化、尾静脉注射、腹腔注射和经鼻吸入等多种途径。

2.1 BLM 单次给药

BLM 单次给药诱导 PF 模型在研究肺纤维化发病机制及筛选潜在抗 PF 有效药物方面具有重要作用[27-28]。但有研究发现,在单剂量 BLM 诱导小鼠 PF 模型中,21 d 后在没有任何干预的情况下,肺纤维化会自发消退[29-30]。

2.1.1 博来霉素单次气管内滴注法

一次性气管内滴注 BLM 是目前国内外最常用的 PF 造模方式,但这种方式也存在药物分布不均匀,进入各肺叶的 BLM 的剂量不同,形成的肺纤维化主要分布在肺门和支气管周围等特点[31-32],常见的气管内滴注法包括气管暴露法和非气管暴露法。

气管暴露法是将动物麻醉后,仰卧位固定于实验台上,无菌条件下进行颈部正中切口,逐层分离软组织,使气管暴露,经气管软骨环间隙进针穿刺向气管内滴注 BLM 溶液,随后缝合切口、消毒。门翔等[33]将 ICR 小鼠麻醉后暴露气管,将 BLM(2.5 mg/kg)溶液经气管滴注入肺部制备 PF 模型,造模干预 28 d 后,小鼠肺功能显著降低,肺泡内出血,肺泡间隔出现大量成纤维细胞,炎症细胞浸润,肺泡壁明显增宽,肺泡间有大量胶原纤维沉积。周飘等[34]通过气管暴露法向 SD 大鼠气管内一次性缓慢注入博来霉素(5 mg/kg)构建 PF 大鼠模型,造模 28 d 后,肺功能降低,肺泡间隔增厚,且在气管和支气管周围的肺组织有大量胶原沉积。气管暴露法可以保证 BLM 完全进入气管,提高了诱导 PF 的准确性,但因其使用外科手术操作导致造模 1 周内动物难以抬头进食,并且增大了动物感染死亡的风险。

非气管暴露法是在动物麻醉后,运用插管装置直接从口腔内经过声门进入气道,然后灌注博来霉素溶液。燕苗苗等[35]采用一次性无创气管内滴注

表 1　各种肺纤维化模型的优点和局限性

Table 1　Advantages and limitations of various pulmonary fibrosis models

造模方式 Moulding methods	优点 Advantages	局限性 Limitations
博来霉素 Bleomycin	造模方式多样、费用低、易于操作，可重复性强，在阐明细胞和分子机制上具有重要作用，能复制出 IPF 典型的肺组织病变 Model has various methods, low cost, easy operation and strong repeatability, which plays an important role in elucidating the cellular and molecular mechanism, and can replicate the typical lung tissue lesions of IPF	缺乏一些 UIP 特征（进行性、不可逆性）、小鼠肺纤维化气管内给药模型中，28 d 后纤维化有一定自限性 Lacking some features of UIP (progressive and irreversible), Some self-limitation of fibrosis after 28 days in an endotracheal administration model of pulmonary fibrosis in mice
石棉 Asbestos	石棉特异性沉积可以诱导出氧化应激和肺泡上皮细胞损伤、能形成持续性的刺激 Asbestos specific deposition can induce oxidative stress and alveolar epithelial cell damage, which can form a persistent stimulation	纤维化常出现在肺中央而不是在胸膜下，纤维化在两肺叶间分布不均匀，且石棉可引起人罕见的间皮瘤 Areas of fibrosis are often central rather than subpleural, fibrosis is unevenly distributed between the lobes, and asbestos can cause mesothelioma, a rare disease in humans
二氧化硅 Silica	产生的纤维化结节类似于职业暴露于粉尘和微粒的肺纤维化患者。二氧化硅难以清除，可形成持久性刺激 Fibrotic nodules resemble pulmonary fibrosis patients with occupational exposure to dust and particulates. Silica is difficult to remove and forms a persistent irritation	缺乏许多间质性肺炎的特征，需要专门的设备来雾化二氧化硅颗粒，且有种属依赖性 Lacking many of the features of interstitial pneumonia. Specialized equipment is required to atomize silica particles and is species dependent
异硫氰酸荧光素 Fluorescein isothiocyanate	免疫荧光确定周围纤维化的沉积区域及肺损伤区域、不依赖于小鼠品系、纤维化持续时间长 Fluorescence identified areas of deposition of surrounding fibrosis and areas of lung injury, independent of mouse strain, and long duration of fibrosis	必须使用新鲜溶液，并在每次使用前进行超声处理，使纤维化的重复性和标准化难以统一、缺乏 UIP 的一些典型特征 Fresh solutions must be used and sonicated prior to each use, making the reproducibility and standardization of fibrosis difficult to unify and lacking some of the typical features of UIP
辐射 Radiation	作为放射性肺炎和纤维化模型的临床意义 Clinical significance as a model of radiation pneumonitis and fibrosis	模型建模周期长、辐射量难以把握、费用较昂贵、并且存在个体抗差异性 Modeling period is long, the amount of radiation is difficult to grasp, the cost is expensive, and there are individual differences in resistance
百草枯 Paraquat	可以诱导出肺泡损伤、氧化应激、内质网应激及细胞因子变化、比较符合百草枯致人肺纤维化病理特点 This model induced alveolar damage, oxidative stress, endoplasmic reticulum stress, and changes in cytokines. It was consistent with the pathological characteristics of paraquat-induced pulmonary fibrosis	造模死亡率高，会引起肺、肝、肾、心脏和中枢神经系统等脏器损伤影响动物状态、缺乏一些 UIP 的典型病理改变 Mortality of UIP model is high, which can cause lung, liver, kidney, heart and central nervous system damage and affect the state of the animal, and lack some typical pathological changes of UIP
病毒载体或转基因动物 Viral vector or transgenic animal	允许有针对性地研究特定的纤维化相关基因、能模拟人类疾病的上皮细胞凋亡和介质变化的特点 Allows targeted studies of specific fibrosis-related genes. Characteristics of epithelial cell apoptosis and mediator changes that mimic human disease	免疫系统会识别并攻击这些载体，使模型转化不理想、具有种属依赖性、费用昂贵 Immune system recognizes and attacks these vectors, making model translation undesirable, species dependent, and expensive

BLM 法对 C57BL/6J 小鼠和 SD 大鼠进行诱导，通过肺功能、肺组织病理、肺组织中 HYP 含量等指标观察，证明一次性气管内滴注 BLM 21 d 均可诱导大、小鼠 PF 模型。Du 等[36]采用一次性无创气管插管滴注 BLM（3 U/kg）建立 SD 大鼠 PF 模型，造模 28 d 后发现，肺组织有较强的实变、肺容量明显降低、肺泡结构紊乱肺间隔增厚、肺组织中胶原沉积及纤维化灶明显增加、肺组织内转化生长因子 β1（transform growth factorβ1，TGF-β1）、α-平滑肌肌动蛋白（alpha-smooth muscle actin，α-SMA）等含量明显增加。非气管暴露法所需设备简单、可重复强，避免了外科手术带来的风险，但由于不能直接观察到 BLM 进入气管，难以保证 100%模型成功率。

2.1.2　博来霉素单次气管内雾化吸入法

气管内雾化吸入法是先将动物麻醉，然后在可视化喉镜操作下将雾化装置经口插入动物气管腔内，使药物在恒定速率下通过气管内雾化形式进入肺脏。王鹤等[37]将 SD 大鼠采用单次气管内雾化 BLM（60 g/L，30 min）方式诱导 PF 模型，28 d 后，肺组织病理可见巨噬细胞、淋巴细胞等浸润，肺泡壁增厚及肺泡间隔增宽，在肺间质区域可见成纤维细胞聚集和胶原基质沉积增加，肺实质结构紊乱，肺组织中羟脯氨酸（hydroxyproline，HYP）、纤溶酶原激活物抑制剂-1（plasminogen activator inhibitor 1，PAI-1）含量显著升高，且与单次气管内滴注组相比动物死亡率更低、肺组织纤维化分布更加均匀弥散。陈广

瑞等[38]将 SD 大鼠进行一次性气管内雾化吸入 BLM(5 mg/kg)诱导 PF 模型,造模后第 21 天,可见肺泡扩张,肺泡壁增厚;肺泡结构紊乱、肺组织实质化、并伴有大量粒细胞浸润;肺泡腔内可见巨噬细胞浸润、胶原增殖显著,成纤维细胞灶分布均匀。Abidi 等[39]首先将 Wistar 大鼠麻醉,然后将 1 个微型喷雾器连接到高压注射器在可视纤维喉镜下经口插入气管腔,使 BLM 溶液以大约 15 μL/s 的速度雾化(颗粒大小:16 ~ 22 μm;操作压力:3000 psi),结果显示在 BLM 诱导第 21 天后,可引起肺间质弥漫性纤维化,表现为肺组织内炎症指数增高、肺纤维化评分升高、肺泡壁明显增厚、肺泡结构紊乱、大量炎性细胞浸润和间质内胶原过度沉积。总之,气管内雾化吸入 BLM 诱导 PF 方式可以使药物在肺内均匀分布,使整个肺组织纤维化病灶更加均匀,有助于更好地表现肺纤维化过程,但其需要专业的气管内雾化设备,造模耗时过长,对于样本量大的实验具有较大挑战性,且雾化过程中药物损耗较大,难以保证真正进入体内的量,造成动物间差异较大,可重复性差。

2.1.3 博来霉素单次尾静脉注射法

BLM 尾静脉给药诱导 PF 动物模型是模拟人体应用药物的方式,其病灶主要位于胸膜下和血管周围,与人 PF 的病理学改变相似。杨聪颖等[40]将 ICR 小鼠经尾静脉一次性注射 200、150、100 mg/kg BLM 建立小鼠 PF 模型,在造模第 14 天后,各剂量组均可见胸膜下及肺血管周围不同程度的炎性改变,肺泡腔及肺间质内可见炎症细胞浸润,部分肺泡腔破坏或消失,肺泡间隔明显增厚,肺组织结构紊乱;在造模后第 14 ~ 21 天各组肺泡炎及肺纤维化程度均显著升高,肺间质内大量炎性细胞浸润,肺实质呈大片融合实变,并最终形成广泛稳定的纤维化,免疫组化结果显示肺组织Ⅲ型胶原面积明显升高,其中以尾静脉一次性注射 BLM 200 mg/kg 后第 21 天诱导建立的 PF 模型成模最好,与人类肺纤维化的病理分布较为接近。涂常力等[41]将 BLM(200 mg/kg)经尾静脉一次性注入 C57BL/6 小鼠体内,在造模后第 4 周,肺 CT 可见周围肺野及肺底网格状阴影,不均匀的斑片状阴影,粗网状不透光影,6 周时肺内病变范围有所减少;肺组织病理显示胸膜下、血管周围、肺泡内和肺泡间隔内不同程度炎性细胞浸润,部分肺泡腔被破坏或结构消失;肺泡间隔不同程度的胶原蛋白沉积,病变区域与正常肺组织的混杂分布(空间异质性);肺组织中Ⅰ型胶原蛋白(collagen-I, COL-Ⅰ)、TGF-β1、α-SMA 及 HYP 蛋白水平均上调。综上所述,单次尾静脉注射 BLM 诱导 PF 模型方式具备操作简便、有效安全方便、与人类肺纤维化的病理接近等优势,但易造成尾部肿胀坏死和胃肠道反应。

2.1.4 博来霉素单次经鼻吸入法

单次经鼻吸入 BLM 诱导 PF 模型是目前国内常用的造模方式[42-44],经鼻吸入法是先将动物麻醉,然后用微量移液器吸取适量 BLM 溶液,将药物经鼻腔缓慢滴入动物气管内,滴入后悬垂旋转。徐钰等[45]将雄性 ICR 小鼠经鼻滴入 BLM(4 mg/kg)诱导 PF 模型,肺病理组织切片显示,在 BLM 刺激后第 7 天,肺泡内大量炎性细胞浸润,无明显的肺纤维化表现;在 BLM 刺激后第 14 天,肺组织可见局灶性纤维组织形成;在 BLM 刺激后第 28 天,肺组织可见广泛纤维化,小鼠肺系数、羟脯氨酸含量、肺泡炎及肺间质纤维化评分均显著升高。杨兴娜等[46]研究发现,SD 大鼠经鼻滴入博来霉素(7 mg/kg)可成功建立 PF 模型,在经鼻滴入 BLM 刺激后第 28 天,肺组织病理显示,肺泡间隔及肺泡内明显炎性反应,局部可见陈旧性出血灶,肺泡腔有大量巨噬细胞浸润,大量胶原纤维沉积,纤维细胞增生明显,肺纤维化及肺泡炎分级明显升高,血清中 MDA 水平明显升高。张秀等[47]将 Wistar 大鼠经鼻一次性滴入 BLM(7 mg/kg)成功制备肺纤维化动物模型,造模后第 28 天,肺组织病理显示肺组织结构严重破坏,细支气管壁上皮细胞水肿、坏死、脱落,管壁纤维化,肺泡腔内见大量炎性细胞浸润,局部Ⅱ型肺泡上皮细胞增生,肺间质纤维组织广泛增生,纤维化区可见大量增生的纤维细胞和胶原纤维沉积,甚至蜂窝样改变;肺组织中 α-SMA、HYP 及转化生长因子 βⅡ型受体含量均显著升高。经鼻吸入诱导 PF 模型具备操作简便、快速、对动物伤害小等优点,但可重复性及造模准确性不高,易造成动物窒息。上述单次诱导模型制作方法和主要指标变化见表 2。

2.2 BLM 多次给药

PF 主要是反复肺泡结构破坏,细胞外基质大量沉积、炎症细胞的浸润以及肺组织异常重构伴蜂窝肺的形成[48-49],为模拟肺泡结构反复破坏的过程,建立更加稳定可靠、病症相似性高的肺纤维化模型,部分研究采用 BLM 多次给药造模方式。

表 2　单次博来霉素诱导的肺部病理和重要指标变化

Table 2　Single bleomycin-induced changes in pulmonary pathology and important indicators

方法 Methods	物种 Species	博来霉素浓度(剂量) Concentration of bleomycin (dose)	肺病理表现 Lung histopathology	主要指标变化 Changes in main indicators	文献 Literatures
气管内滴注 Tracheal perfusion	ICR 小鼠 ICR mice	2.5 mg/kg (0.1 mL)	造模后第 28 天肺泡内出血,肺泡间隔出现大量纤维细胞,炎症细胞浸润;肺泡壁明显增宽,肺泡间有大量胶原纤维沉积 On the 28th day after modeling, alveolar hemorrhage, a large number of fibrocytes and inflammatory cell infiltration appeared in the alveolar septum. The alveolar wall was significantly widened and there was a large amount of collagen fiber deposition between the alveoli	FVC ↓、FEV0.1 ↓、TNF-α ↑、IL-6 ↑、IL-8 ↑、HYP ↑、TGF-β1 ↑	[33]
	SD 大鼠 SD rat	5 mg/kg	造模后第 28 天肺泡间隔增厚,且在气管和支气管周围的肺组织有大量胶原沉积 On the 28th day after modeling, the alveolar septum was thickened, and a large amount of collagen was deposited in the lung tissue around the trachea and bronchus	Cydn ↓、COL1A1 mRNA ↑、RL ↑、Re ↑	[34]
	C57BL/6J 小鼠 C57BL/6J mice	5 mg/kg	造模后第 21 天肺组织结构破坏、肺泡壁明显增厚,全肺产生炎性浸润;肺组织明显纤维条索形成及胶原沉积 On the 21st day after modeling, the lung tissue structure was destroyed, the alveolar wall was significantly thickened, and the whole lung was inflammatory infiltration. The lung tissue showed obvious fibrous cord formation and collagen deposition	HYP ↑;TV ↓、MV ↓	[35]
	SD 大鼠 SD rat	3 U/kg	造模后第 28 天,模型组大肺组织均有较强的实变、肺容量明显降低、肺泡结构紊乱肺间隔增厚、肺组织中胶原沉积及纤维化灶明显增加 On the 28th day after modeling, the large lung tissue of the model group had strong consolidation, significantly reduced lung volume, disordered alveolar structure, thickened lung septum, and significantly increased collagen deposition and fibrosis foci in lung tissue	TGF-β1 ↑、α-SMA ↑、TNF-α ↑、IL-1β ↑、IL-6 ↑	[36]
	SD 大鼠 SD rat	60 g/L (30 min)	造模后第 28 天,肺组织病理可见巨噬细胞、淋巴细胞等浸润,肺泡壁增厚及肺泡间隔增宽,在肺间质区域可见成纤维细胞聚集和胶原基质沉积增加,肺实质结构紊乱 After 28 days of modeling, the lung tissue displayed pathological changes characterized by infiltration of macrophages and lymphocytes, thickening of the alveolar walls and widening of the alveolar septa, aggregation of fibroblasts, increased deposition of collagen matrix in the interstitial area of the lung, and structural disorder in the interstitial area of the lung parenchyma	HYP ↑、PAI-1 ↑	[37]
气管内雾化 Aerosol inhalation	SD 大鼠 SD rat	5 mg/kg (15 s)	造模后第 21 天,肺泡结构改变、肺组织实质化,并伴有多量粒细胞浸润、肺泡腔内可见巨噬细胞浸润、肺组织损伤明显、胶原增殖显著 On the 21st day after modeling, alveolar structure changed, lung tissue was parenchymal, accompanied by a large number of granulocyte infiltration, macrophage infiltration in alveolar space, obvious lung tissue injury, and significantly increased collagen	HYP ↑、TGF-β1 ↑	[38]
	Wistar 大鼠 Wister rat	2 mg/kg	造模后第 21 天肺组织内炎症指数增高、肺纤维化评分升高、肺泡壁明显增厚、肺泡结构紊乱、大量炎性细胞浸润和间质内胶原过度沉积 On the 21st day after modeling, the inflammatory index and pulmonary fibrosis score in lung tissue were increased, the alveolar wall was significantly thickened, the alveolar structure was disordered, a large number of inflammatory cell infiltration and excessive deposition of collagen in the interstitium were found	TGF-β ↑、MDA ↑、Catalase ↓、SOD ↓	[39]

续表 2

方法 Methods	物种 Species	博来霉素浓度(剂量) Concentration of bleomycin (dose)	肺病理表现 Lung histopathology	主要指标变化 Changes in main indicators	文献 Literatures
尾静脉注射 Tail vein injection	ICR 小鼠 ICR mice	200、150、100 mg/kg	造模后第 21 天,肺实质呈大片融合实变,其间有大量的炎症细胞浸润,并最终形成广泛稳定的纤维化 After 21 days of modeling, the lung parenchyma exhibited a significant area of fusion and consolidation, accompanied by a substantial infiltration of inflammatory cells, ultimately resulting in extensive and stable fibrosis	COL-Ⅲ ↑	[40]
	C57BL/6 小鼠 C57BL/6 mice	200 mg/kg	造模后 2 周,肺组织出现大量炎症细胞浸润;4 周可见广泛纤维化病变,部分可见小灶分布的成纤维细胞灶;6 周时肺纤维化病变较前减轻 At 2 weeks after modeling, a large number of inflammatory cells infiltrated in the lung tissue. At 4 weeks, extensive fibrosis and small foci of fibroblasts were observed. At 6 weeks, pulmonary fibrosis was alleviated	COL-Ⅰ ↑、TGF-β1 ↑、α-SMA ↑	[41]
	ICR 小鼠 ICR mice	4 mg/kg	造模第 7 天后,肺泡内大量炎性细胞浸润,无明显的肺纤维化表现;造模 14 天后,肺组织可见局灶性纤维组织形成;造模 28 天后,肺组织可见广泛纤维化,肺泡炎及肺间质纤维化评分均显著升高 After 7 days of modeling, a large number of inflammatory cells infiltrated in the alveoli, and there was no obvious pulmonary fibrosis. After 14 days of modeling, focal fibrous tissue formation was observed in lung tissue. After 28 days of modeling, extensive fibrosis was observed in lung tissue, and the scores of alveolitis and pulmonary interstitial fibrosis were significantly increased	HYP ↑	[45]
经鼻吸入 Intranasal drip	SD 大鼠 SD rat	7 mg/kg	造模后第 28 天,肺泡间隔及肺泡内明显炎性反应,局部可见陈旧性出血灶,肺泡腔大量巨噬细胞浸润,大量胶原纤维沉积,纤维细胞增生明显,肺纤维化及肺泡炎分级明显升高 On the 28th day after modeling, there were obvious inflammatory reactions in alveolar septum and alveoli, local old hemorrhage foci, a large number of macrophages infiltration in alveolar cavity, a large number of collagen fibers deposition, and obvious fibroblast proliferation, and the grade of pulmonary fibrosis and alveolitis was significantly increased	MDA ↑	[46]
	Wistar 大鼠 Wister rat	7 mg/kg	造模第 28 天后,肺组织结构严重破坏,细支气管壁上皮细胞水肿、坏死、脱落,管壁纤维化,肺泡腔内见大量炎性细胞浸润,局部Ⅱ型肺泡上皮细胞增生,肺间质纤维组织广泛增生,纤维化区可见大量增生的纤维细胞和胶原纤维沉积,甚至蜂窝样改变 After 28 days of modeling, the structure of lung tissue was severely damaged, with edema, necrosis, and shedding of bronchiolar epithelial cells, fibrosis of the wall, a large number of inflammatory cells infiltration in the alveolar cavity, local proliferation of type Ⅱ alveolar epithelial cells, extensive proliferation of pulmonary interstitial fibrous tissue, and a large number of proliferative fibroblasts and collagen fibers deposition in the fibrotic area, even honeycomb-like changes	α-SMA ↑、HYP ↑、Smad4 ↑、TGF-βRⅡ ↑、Smad2 ↑、p-Smad2 ↑、Smad7 ↓	[47]

2.2.1 博来霉素多次气管内滴注法

陈孟毅等[50]将 C57BL/6J 小鼠无创气管内滴注 BLM(0.4 mg/mL,每只 100 μL),2 周诱导 1 次,共 8 次,给药后 0.5、1、2、4 个月时肺组织肺泡腔胞浆渗出逐渐减少,血管周围炎细胞浸润,肺泡间隔增宽,其中以 BLM 诱导 0.5 个月时程度最明显。同时,胶原纤维在 0.5 个月时出现,且在继续给药 1、2、4 个月时胶原纤维持续存在;在给药 4 个月时肺组织胶原纤维和衰老细胞 β-半乳糖苷酶明显增加。Redente 等[51]将 C57BL/6J 小鼠通过无创气管内重复滴注 BLM 制备 PF 模型,经过 3 次双周 BLM (1 U/kg) 诱导后,在 6 ~ 24 周表现出持续性和进

行性肺纤维化,肺组织病理显示胶原沉积增加、成纤维细胞积聚、纤维化区域内Ⅰ型和Ⅱ型肺泡上皮细胞丢失、club 细胞分泌蛋白(club cell secretory protein, CCSP)使细支气管肺实质化、肺组织蜂窝样改变,以及增生性 KRT8 上皮细胞的过度积累,显微计算机断层扫描成像显示明显的牵引性支气管扩张和胸膜下纤维化。上述研究表明,多次气管内滴注 BLM 诱导 PF 方式能更充分再现 PF 患者的肺上皮重塑,模拟持续性和进行性肺纤维化,但存在动物死亡率高、造模时间耗时过长、经济代价高等缺点。

2.2.2　博来霉素多次气管内雾化吸入法

多次气管内雾化吸入 BLM 是近年来探究 PF 发病机制及筛选有效防治药物开展的一种造模方式。王鹤等[37]将 SD 大鼠以多次雾化吸入 BLM (60 g/L)30 min, 连续雾化 3 d,诱导 PF 模型,造模 28 d 后,肺组织病理显示肺间隔中、重度增厚、肺间质慢性活动性炎症、间质可见成团的纤维组织增生、甚至肺泡结构消失,形成连续的纤维化;大鼠肺组织中 HYP 及 PAI-1 含量均显著升高。多次雾化吸入 BLM 制备的 PF 模型,其肺病理损伤和生理指标相对稳定,符合肺纤维化的演变过程,是一种有效的 PF 模型制备手段,但由于这种造模方式运用较少,需进一步扩大样本量以明确具体的造模效果。

2.2.3　博来霉素多次腹腔内注射法

腹腔注射 BLM 诱导 PF 模型方式通常需要多次注射,其纤维化病灶主要位于胸膜下和血管周围,与人 PF 病理改变相似[52-53],是近年来比较常用的造模方式。苏敏红等[54]将 C57BL/6 小鼠分别通过每周 1 次和 2 次腹腔注射 BLM(35 mg/kg),共 8 次,诱导 PF 模型,两组在第 8 次腹腔注射后 2、4、6、8、10 周均出现不同程度的肺泡炎、肺纤维化;免疫组织化学显示 COL-Ⅰ主要沉积在胸膜下、血管周围肺间质和肺泡间隔;造模 2 周后肺组织中 COL-Ⅰ、α-SMA、TGF-β1 及 HYP 含量均显著增高,并持续至第 10 周,其中每周 2 次腹腔注射 BLM 诱导 PF 模型稳定性更好。Liu 等[55]将 C57BL/6 小鼠分别于第 1、5、8、11、15 天腹腔注射 BLM(5 mg/mL,每只 100 μL)建立 PF 模型,在造模后第 28 天,肺病理组织显示弥漫性肺泡损伤、肺泡间隔水肿、明显纤维化伴有肺组织结构破坏和广泛胶原沉积,肺组织中 TGF-β1、COL-Ⅰ及 α-SMA 含量均显著升高。程雪等[56]将 C57BL/6 小鼠腹腔注射 BLM (7.5 mg/(kg·d)),连续 10 d,制备 PF 模型,造模后第 28 天,肺组织病理显示肺泡间隔明显增厚,肺泡间隔和肺泡腔有大量炎症细胞浸润及大量胶原纤维渗出,肺泡炎及肺纤维化评分显著升高;血清中 TGF-β1、肿瘤坏死因子 α(tumor necrosis factor alpha, TNF-α)及肺组织中 α-SMA、COL-Ⅰ、Ⅲ型胶原蛋白(collgen-Ⅲ, COL-Ⅲ)的含量均明显升高。结合以上结果,多次腹腔内注射 BLM 方式诱导 PF 模型具备操作简单、病灶分布较均匀,与临床 PF 患者病理分布相似等优点,但由于腹腔注射 BLM 给药剂量和频率多样,模型稳定性各异,且所需药量较大,易导致腹腔内其他器官损伤和胃肠道反应,影响广泛推广应用。

2.2.4　博来霉素多次尾静脉注射法

近年来小剂量多次尾静脉注射 BLM 诱导 PF 模型被用来探索纤维化发病机制的研究屡见报道[41,57]。孟婕等[58]通过小剂量多次尾静脉注射 BLM 方式诱导 PF 模型,实验中将 ICR 小鼠放入固定器内固定,露出尾巴,用 75%乙醇消毒,显露尾静脉,1 mL 注射器按 10 mg/kg 给予 BLM 注射,持续 14 d,造模第 28 天后,肺组织病理显示肺组织大片融合实变,肺泡及肺间隔内可见巨噬细胞和淋巴细胞渗出,成纤维细胞增生,肺泡结构紊乱;肺泡间隔及肺间质大量胶原纤维沉积,纤维化病灶主要聚集在胸膜下并均匀分布于肺间质;肺组织中 HYP 含量及肺间质损伤指数均显著升高。Gul 等[59]将 C57BL/6 小鼠分为低剂量、高剂量组,分别以 10 mg/kg 和 20 mg/kg 尾静脉注射 BLM,连续 7 d,制备 PF 模型。造模第 28 天后,小鼠肺功能明显下降;PET/CT 显像及分析显示肺纤维化实变、非通气肺面积及高密度肺面积均明显增加;肺泡灌洗液和血清中 TGF-β1、TNF-α、IL-6、GM-CSF 及肺组织中 HYP、PAI-1 含量均显著性升高;肺病理显示肺泡结构破坏、纤维化面积及胶原沉积明显增加,肺纤维化的发生集中于胸膜下,均匀分布于肺间质;透射电镜超微结构图象可见Ⅰ型肺泡上皮细胞变性、崩解、脱落,内皮细胞肿胀,Ⅱ型肺泡上皮细胞增生,粗面内质网扩张,胶原纤维在间质内大量堆积。综上所述,小剂量多次尾静脉注射 BLM 诱导 PF 模型接近人类 PF 疾病特征,但易造成动物尾部肿胀、溃烂坏死和胃肠道反应。上述多次诱导模型制作方法和主要指标变化见表 3。

表 3 多次博来霉素诱导的肺部病理和重要指标变化

Table 3 Multiple bleomycin-induced changes in pulmonary pathology and important indicators

方法 Methods	物种 Species	博来霉素浓度（剂量） Concentration of bleomycin (dose)	肺病理表现 Lung histopathology	主要指标变化 Changes in main indicators	文献 Literatures
气管内滴注 Tracheal perfusion	C57BL/6J 小鼠 C57BL/6J mice	0.4 mg/mL（每只 100 μL），2 周给药 1 次，共 8 次 0.4 mg/mL (100 μL per mouse), once every 2 weeks, a total of 8 times	造模后 0.5、1、2、4 个月时肺组织肺泡腔胞浆渗出逐渐减少，血管周围炎细胞浸润，肺泡间隔增宽，其中以 BLM 诱导 0.5 个月时程度最明显。肺组织中胶原纤维增加 At 0.5, 1, 2 and 4 months after modeling, the alveolar cytoplasmic exudation gradually decreased, perivascular inflammatory cells infiltrated, and alveolar septum widened, which was the most obvious at 0.5 months after BLM induction. Collagen fibers were increased in lung tissue	SA-β-Gal ↑	[50]
	C57BL/6J 小鼠 C57BL/6J mice	1 U/kg，2 周给药 1 次，共 3 次 1 U/kg, once every 2 weeks, a total of 3 times	造模后 6 ~ 24 周之间表现出持续性和进行性肺纤维化，肺组织病理显示胶原沉积增加、成纤维细胞积聚、纤维化区域内Ⅰ型和Ⅱ型肺泡上皮细胞丢失、肺实质内 CCSP 细胞的细支气管化、肺组织蜂窝样改变 Persistent and progressive pulmonary fibrosis was observed between 6 and 24 weeks after modeling. Lung histopathology showed increased collagen deposition, accumulation of fibroblasts, loss of type Ⅰ and type Ⅱ alveolar epithelial cells in the fibrotic area, bronchiolitization of CCSP cells in lung parenchyma and honeycomb-like changes in lung tissue.	α-SMA ↑、HYP ↑、TGF-β ↑、Fibroblasts ↑	[51]
气管内雾化 Aerosol inhalation	SD 大鼠 SD rat	60 g/L，30 min，雾化 3 d 60 g/L, 30 min, continuous aerosol inhalation for 3 d	造模第 28 天后，肺组织病理显示肺间隔中、重度增厚、肺间质慢性活动性炎症、间质可见成团的纤维组织增生、甚至肺泡结构消失，形成连续的纤维化 On the 28th day after modeling, lung tissue pathology showed moderate to severe thickening of the pulmonary septum, chronic active inflammation in the pulmonary interstitium, mass fibrous tissue proliferation in the interstitium, and even disappearance of alveolar structure, forming continuous fibrosis	HYP ↑、PAI-1 ↑	[37]
	C75BL/6 小鼠 C57BL/6 mice	35 mg/kg，2 次/周，共注射 8 次 35 mg/kg, twice a week, with a total of 8 injections	在造模后 2 ~ 10 周，肺组织血管周围、肺泡腔内、肺泡间隔、胸膜下均有不同程度的炎性细胞浸润，以淋巴细胞为主，肺泡间隔增厚，肺泡腔内有大量胶原纤维渗出，肺泡腔扩大，肺泡壁变薄，肺泡结构被破坏消失；肺泡间隔大量胶原蛋白沉积 From 2 to 10 weeks after modeling, there were varying degrees of inflammatory cell infiltration around blood vessels, alveolar cavity, alveolar septum and subpleura in lung tissue, mainly lymphocytes, thickened alveolar septum, a large number of collagen fibers exudate in alveolar cavity, alveolar cavity expanded, alveolar wall became thinner, alveolar structure was destroyed and disappeared. A large amount of collagen was deposited in the alveolar septum	COL-Ⅰ ↑、α-SMA ↑、HYP ↑、TGF-β1 ↑	[54]
腹腔内注射 Intraperitoneal injection	C75BL/6 小鼠 C57BL/6 mice	5 mg/mL（100 μL），第 1、5、8、11、15 天腹腔注射 5 mg/mL (100 μL), intraperitoneal injection on the 1st, 5th, 8th, 11th and 15th day	在 BLM 给药后第 28 天，肺组织弥漫性肺泡损伤、肺泡间隔水肿、明显纤维化伴有肺组织结构破坏和广泛胶原沉积 On the 28th day after BLM administration, diffuse alveolar injury, edema of alveolar septa, obvious fibrosis accompanied by structural destruction of lung tissue and extensive collagen deposition occurred in lung tissue	COL-Ⅰ ↑、α-SMA ↑、TGF-β1 ↑、p-Smad2 ↑、p-Smad3 ↑、E-cadherin ↓	[55]
	C75BL/6 小鼠 C57BL/6 mice	7.5 mg/(kg·d)，连续 10 d 7.5mg/(kg·d) for 10 days	造模后第 28 天，模型组肺泡间隔明显增厚，肺泡间隔和肺泡腔有大量炎症细胞浸润及大量胶原纤维渗出，肺泡炎及肺纤维化评分显著升高 On the 28th day after modeling, the alveolar septum of the model group was significantly thickened, with a large number of inflammatory cells infiltration and collagen fiber exudation in the alveolar septum and alveolar cavity, and the scores of alveolitis and pulmonary fibrosis were significantly increased	COL-Ⅰ ↑、α-SMA ↑、COL-Ⅲ ↑、TGF-β1 ↑、TNF-α ↑	[56]

续表 3

方法 Methods	物种 Species	博来霉素浓度（剂量） Concentration of bleomycin (dose)	肺病理表现 Lung histopathology	主要指标变化 Changes in main indicators	文献 Literatures
尾静脉注射 Tail vein Injection	ICR 小鼠 ICR mice	10 mg/kg，每天 1 次，持续 14 d 10 mg/kg once a day for 14 days	在 BLM 给药后第 28 天后，肺组织病理显示肺组织大片融合实变，肺泡及肺间隔内可见巨噬细胞和淋巴细胞渗出，成纤维细胞增生，肺泡结构紊乱，Masson 染色可见肺泡间隔及肺间质大量胶原纤维沉积 After 28th days of BLM administration, pathological examination of lung tissue revealed extensive fusion and consolidation, exudation of macrophages and lymphocytes, hyperplasia of fibroblasts, as well as disorder in alveolar septum and interstitial lung structure. Masson staining demonstrated significant deposition of collagen fibers in the alveolar septum and interstitial lung.	HYP ↑	[58]
	C75BL/6 小鼠 C57BL/6 mice	10 mg/kg 20 mg/kg，每天 1 次连续 7 d 10 mg/kg 20 mg/kg, once a day for 7 days	造模第 28 天后，肺病理显示肺泡结构破坏、纤维化面积及胶原沉积明显增加，肺纤维化的发生集中于胸膜下，均匀分布于肺间质 After 28th days of modeling, the pathological film of lung tissue showed that the destruction of alveolar structure, fibrosis area and collagen deposition increased significantly. The occurrence of pulmonary fibrosis was concentrated in the subpleura and evenly distributed in the lung interstitium	TGF-β1 ↑、TNF-α ↑、IL-6 ↑、HYP ↑、PAI-1 ↑	[59]

表 4　博来霉素不同给药方式的优点和局限性

Table 4　Advantages and limitations of different delivery methods of bleomycin

给药方式 Drug-delivery way	优点 Advantages	局限性 Limitations
气管内滴注 Tracheal perfusion	造模时间短、费用较低、可重复性高、成模周期短、仪器工具简单易得，能复制出 IPF 典型的肺组织病变 Short modeling time, low cost, high repeatability, short modeling cycle, and easy access to instruments and tools. It can replicate the typical lung tissue lesions of IPF	对操作者技术要求较高，肺纤维化集中在肺门和支气管周围，并且病变在不同肺叶分布不均匀，单次给药存在肺纤维化的自限性 High technical requirements for operators. Pulmonary fibrosis is concentrated around the hilum and bronchus, and the lesions are unevenly distributed in different lung lobes. There was self-limitation of pulmonary fibrosis with a single dose
气管内雾化 Aerosol inhalation	药物在体内均匀分布，使整个肺组织均匀肺纤维化 Drug is evenly distributed in the body and the whole lung tissue is evenly fibrosed	需要专业的动物用气管内雾化设备，造价昂贵，造模耗时过长，不适用于需要样本量大的实验 Professional equipment for animal endotracheal atomization is required. Expensive、long time, not suitable for experiments requiring large sample sizes
尾静脉注射 Tail vein injection	实验条件易于控制，重复性好，病理及相关细胞因子改变与临床 IPF 相似 Experimental conditions were easy to control and reproducible. The pathological changes and related cytokines were similar to those in clinical IPF	操作难度较大，易造成尾部肿胀坏死和胃肠道反应，影响动物生存状态，且成模效果存在较大差异 This method is difficult to operate, easy to cause tail swelling and necrosis and gastrointestinal reaction, affecting the survival state of animals, and there are great differences in the modeling effect
经鼻吸入 Inhaled by nose	操作简便、快速，动物损伤小 Easy and fast operation, little animal damage	可操作性、可重复性及造模准确性不高，易造成动物窒息 Low operability, reproducibility and accuracy of mold making, easily causing animal suffocation
腹腔注射 Intraperitoneal injection	可减轻因手术操作差异而引起的纤维化程度，操作简单、病灶分布较匀、纤维化区主要集中在胸膜下，与临床 IPF 病理分布相似 Can reduce the degree of fibrosis caused by differences in surgical procedures. Operation is simple, the distribution of lesions is uniform, and the fibrotic area is mainly concentrated in the subpleura, which is similar to the pathological distribution of clinical IPF	需多次给药，所需药量较大，易导致腹腔内其他器官损伤和胃肠道反应 Multiple administrations are required, requiring larger amounts of drugs, causes damage to other organs in the abdominal cavity and gastrointestinal reactions

3 结语与展望

PF 是一种病因不明的致命性疾病,可靠的肺纤维化动物模型在探索其发病机制、探寻有效治疗药物中具有重要作用。通过总结近 20 年相关文献发现:(1)BLM 为 PF 模型建立的常用诱导剂。(2)在动物选择方面,以鼠尤为广泛,包括 Wistar 大鼠、SD 大鼠、BALA/C 小鼠、C57BL/6 小鼠、KM 小鼠和 ICR 小鼠等。其中,C57BL/6 小鼠和 Wistar 大鼠更容易复制出 PF 模型[60-61]。(3)在诱导方式上有气管内滴注、气管内雾化、尾静脉注射、腹腔注射和经鼻吸入,以气管内滴注尤为常见。不同诱导方式各有特点(见表 4)。(4)在诱导频次上,有单次和多次,多次诱导有连续给药、每周 2 次给药及其他间断给药等。BLM 单次给药诱导 PF 模型应用较为广泛,因其操作简便、成模较快,病理表现典型,在阐明 PF 机制研究上具有重要作用[62],但并不能模拟出人 PF 进行性、不可逆性等重要特征[53]。BLM 多次给药方式能复制出人 PF 进程中慢性、进行性的肺损伤和明显的肺泡上皮细胞增生,且产生的炎性反应较弱,可造成更持久的纤维化[63],这对于研究上皮间质转化、肺重塑与修复具有重要意义,但该方式经济成本高、造模时间久、对动物创伤较大。

目前的模型研究仍具有一些局限性,如:BLM 来源不一、实验条件不同、观察时间不统一、缺乏高质量的模型依据、部分研究存在盲目仿制等。因此,未来研究中应注意:(1)在模型的建立上,明确 BLM 的来源、动物的种属以及模型稳定性的探索,建立稳定、可重复性强、更为符合人类疾病进程及病理特征的肺纤维化动物模型。(2)在研究中应合理选择模型:对于疾病机制研究可不局限于某种特定模型,对于药效评价及作用机制研究需根据药物的疗程,疗程短的可以选取单次诱导模型,对于治疗周期长或需要评价远后效应的研究需选取模型持续时间长的诱导方式。

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