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铜绿假单胞菌肺部感染动物模型

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摘　要

铜绿假单胞菌是一种具有很强抗生素耐药性的条件致病菌，能够引起广泛的危及生命的急性和慢性感染。它是细菌性肺部感染的主要病原菌之一，具有很高的发病率和死亡率。建立有效、合适的动物模型能够极大地帮助我们了解疾病的发病机制以及测试和开发新的治疗策略。本文旨在通过对以铜绿假单胞菌为病原菌建立的动物肺炎模型方法的概述及评价，为基础与临床研究应用提供理论基础。

关键词

铜绿假单胞菌，急性感染，慢性感染，动物模型

Animal Models of *Pseudomonas aeruginosa* Lung Infection

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Abstract

***Pseudomonas aeruginosa* is a highly antibiotic-resistant conditionally pathogenic bacterium capable of causing a wide range of life-threatening acute and chronic infections. It is one of the major pathogens of bacterial lung infections with high morbidity and mortality. The establishment of effective and appropriate animal models can greatly assist our understanding of disease pathogenesis as well as testing and developing new therapeutic strategies. The aim of this paper is to provide a theoretical basis for basic and clinical research applications by detailing and evaluating the methodology of animal pneumonia models established using *Pseudomonas aeruginosa* as the causative agent.**

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Keywords

***Pseudomonas aeruginosa*, Acute Infection, Chronic Infection, Animal Model**

1. 引言

1.1. 呼吸道防御作用

呼吸过程中，空气中的尘埃及病原体如细菌和病毒等微生物会持续经由呼吸道吸入肺部[1]。为保护呼吸道免受侵入细菌的感染，呼吸道上皮层，主要组成为假复层纤毛柱状上皮细胞，藉由紧密连接，将细胞顶膜侧完整密封，形成一道物理屏障，防止离子、水分和病原微生物通过[2] [3]。上皮层中，夹藏其他多种功能不同的细胞，包括杯状细胞[4]、俱乐部细胞(Club cells) [5]等，其下方又含有基底细胞和粘膜下腺[6] [7]。上皮细胞层表面覆盖有一层溶液层，称之为表面液体(Airway surface liquid, ASL)，其包含上层凝胶状的粘液层(Mucus layer, MCL)和下层胶状的纤毛旁液体层(Periciliary liquid, PCL) [2] [8] [9]。粘液是由杯状细胞和粘膜下腺分泌出来，在粘液层可用来黏附呼吸道中的病原体和尘埃，而下方的纤毛旁液体层纤毛快速节律的摆动可移动粘液，朝着口腔方向输送，形成痰液而排出[2] [8] [9] [10]。这一过程称之为粘液纤毛清除(Mucociliary clearance, MCC)防御机制[9] [10]。

纤毛旁液体层也具有许多抗菌物质，由上皮细胞和细胞分泌，包括防御素、溶菌酶、乳铁蛋白、抗菌肽、免疫调节分子(如细胞因子)等[11] [12]。抗菌肽和抗菌蛋白可直接识别细菌并裂解细菌膜，从而在几秒钟到几分钟内杀死细菌[13]。例如 Enrico F Semeraro 等[14]人发现，乳铁蛋白可在几秒钟内损害大肠杆菌的细胞质膜，并在细胞溶质中以高浓度积累最终将细菌杀死。许多肺部疾病是由于呼吸道纤毛的损伤，粘液纤毛清除吸入病原微生物的能力降低，从而使得残存的病原微生物可定植和感染上皮细胞层，最终导致肺部炎症反应[2] [8] [10]。

存留呼吸道的细菌，一旦逃脱上皮细胞层的清除机制，可能会引起许多相关的疾病如支气管炎(Bronchitis) [15] [16]、慢性阻塞性肺疾病(Chronic obstructive pulmonary disease, COPD) [17] [18] [19]和囊性纤维化肺疾病(Cystic fibrosis, CF) [18] [19] [20]等。这些呼吸系统疾病在中国乃至全球都是危害健康和生命的主要原因[18] [19]。

在治疗细菌性肺炎感染时，由于感染源菌种的鉴定费时，且慢性肺炎常伴随着多种细菌定植于患者的肺中，所以治疗早期多是联合使用多种抗生素[21] [22]。但抗生素治疗速度慢且成本高[23]，也衍生出因频繁使用抗生素而产生耐药菌种的问题[24] [25]，从而造成更大的医疗和经济负担[25]。因此，经由探究细菌侵染的过程及致病机理，找出可能的治疗靶点，因而研发针对细菌性感染新的治疗方法是目前研究热点[26]。

1.2. 细菌侵染

细菌逃避宿主的杀菌机制后，会留存于纤毛旁液体层，首先附着(Adherence)在呼吸道表面[27]，借助鞭毛的运动、流体动力、布朗运动等方式到达呼吸道表面[28]，并通过粘附素(Adhesin) (如菌毛(Pili))通过与宿主表面相应受体相互作用，从而促进细菌黏附到呼吸道粘膜表面[29] [30] [31]。细菌附着到上皮组织

后与上皮细胞表层交互作用，产生黏附结合[32]。此外，菌毛与其他细菌的菌毛相互作用可促进更多细菌结合，造成细菌聚集形成菌群并开始增殖[27] [29]。细菌间通过群体感应(Quorum sensing, QS)机制，释放感应物质如酰基高丝氨酸内酯(Acylhomoserine lactones, AHLs)，实现细菌群间的通信[33]，可促进细菌间合成和分泌胞外多糖聚合物基质(Extracellular polymeric substances, EPS)，形成生物膜(Biofilms) [29] [30]。生物膜通过中和抗菌剂或限制扩散来减少抗生素的影响[29]，及提高对药物作用的抵抗力[34]。这些定植的细菌主要可通过两种方式侵染上皮细胞层，第一是直接经由内化方式进入上皮细胞，再破坏细胞膜而进入上皮层下方[35]。第二种方式是经由解离上皮层细胞与细胞间的紧密连接，达到相同的效果[35] [36]。穿过上皮层后，细菌可获取大量养分，快速增长且引发严重的免疫反应[31]。这是造成肺部炎症反应和后续肺水肿[37]及败血症[38]等与致死相关疾病的主因。

1.3. 细菌侵染实验模型

为了探究上述细菌侵染上皮细胞层之后，炎症发生的机制，及相关药物的作用和原理，许多实验模型，包括细胞系培养[39] [40] [41] [42]，原代细胞培养形成上皮细胞层的组织模型[40] [43] [44] [45]，及实验动物[46] [47] [48] [49]已被广泛采用。细胞培养模型有助于我们了解细菌感染对细胞产生作用的分子机制[42] [44] [45]，培养的上皮细胞层和动物模型则可模拟细菌感染生物体的过程，而动物模型可进一步观察病理生理的变化，炎症反应发生的过程，及对药物治疗的直接效用[50] [51] [52]。

本综述将针对肺部感染常见的铜绿假单胞菌[53] [54] [55]，阐述目前常用的急性和慢性感染动物模型的建立方法和应用，并总结相关研究对该细菌入侵上皮细胞机制的进展。

2. 铜绿假单胞菌感染的动物模型

2.1. 铜绿假单胞菌的临床重要性

铜绿假单胞菌(*Pseudomonas aeruginosa*)是一种绿色、短杆状的革兰氏阴性细菌病原体[53] [56]，存在于广泛自然环境中，具有很强的抗生素耐药性。在宿主防御功能受损时，如中性粒细胞减少症、严重烧伤、支气管扩张或囊性纤维化遗传疾病[57] [58] [59]，会引起严重的急性和慢性感染[60] [61]，其死亡率高达 40% [60]，所以常描述其为一种条件致病菌(Opportunistic pathogen)。也由于其耐药性强，可生存于医疗设施的表面[62]，所以是引起医院感染的主因之一。

铜绿假单胞菌常见于肺炎、手术部位感染、尿路感染和菌血症等[53] [63]。由于铜绿假单胞菌的耐药性高，感染后，抗生素治疗也往往不容易根除[62]。Moore & Flaws 等[64]人研究发现，尽管使用了联合药物治疗法，但铜绿假单胞菌的耐药率仍在上升。也由于铜绿假单胞菌的耐药性强，其也是许多肺部慢性感染疾病如囊性纤维化肺疾病(Cystic fibrosis, CF)、慢性阻塞性肺疾病(Chronic obstructive pulmonary disease, COPD)的主要感染源之一[61] [62]。因此，铜绿假单胞菌的感染已成为一个全球性的医疗问题[65]。

临床的治疗方案，主要是通过使用抗生素如 β-内酰胺类抗生素、氟喹诺酮、四环素、多粘菌素等进行治疗[60]。但由于铜绿假单胞菌已衍生出广泛的耐药菌株，其治疗方案日益受到限制[62] [66]。因此，为解决抗生素治疗衍生的问题及减少对其的依赖性，了解细菌感染肺部的过程及细菌对宿主呼吸道上皮细胞层的侵染机制，则有助于开发新的治疗方式或改善目前的治疗方法[67]。

2.2 铜绿假单胞菌感染的动物模型的建立

探讨铜绿假单胞菌感染的动物模型主要为小鼠或大鼠[60]，而其他动物，如兔子[68] [69]、猪[70]、狗[71]、猫[72]等也有被采用。动物感染实验可分为急性和慢性感染[61]，其中急性感染通过短时接种大量的细菌，在小鼠和大鼠中约(1×10^5~1×10^{11} CFU/ml)进行处理，处理时间大约 24 小时到 72 小时[69] [73]

[74] [75]；在慢性感染中，小鼠和大鼠接种细菌量约(2×10^6~1.0×10^{11} CFU/ml)，处理时间相对较长，大约 7 天至几个星期[76] [77] [78]。值得注意的是，研究不同动物之间，给予感染的细菌量需进行相对应调整[78] [79]。

细菌感染的动物模型，主要有 4 个探究目的：1) 细菌感染引起呼吸道免疫反应的机制[69] [73] [74]；2) 抗生素或抗炎药物的作用[80] [81]；3) 临床的药物治疗及剂量优化[80] [81]；4) 探究细菌接种量及感染方式对呼吸道产生的毒力作用及致病机制[69] [73] [82]。

2.3 细菌感染方式

为了达到上述研究目的及建立相关急性和慢性感染的动物模型，主要有三种细菌给予方式：气管插管[73] [74]、鼻滴注[82] [83]和雾化吸入[84] [85]。不同的接种方式可能影响细菌进入到呼吸道的位置，其研究重点也随之不同，对动物模型的建立也有很大的差异。下面将总结基于这三种细菌给予方式而建立的急性和慢性动物感染模型。

1) 气管插管

气管插管法主要通过短暂麻醉动物如小鼠和大鼠后，手术暴露气管进行气管插管，然后将细菌悬浮液或包裹细菌的琼脂珠注入到气管或肺部，之后进行手术缝合[73] [80] [81] [86]。手术后观察动物并按预先处理时间进行动物处理[73] [81]。气管插管法常用于急性和慢性感染，其中慢性感染主要使用包裹有铜绿假单胞菌的琼脂珠进行感染[86]，其目的是将细菌物理性地保留在气道中[87]。值得注意的是，无菌的琼脂珠本身可能会刺激引发炎症反应，可能会降低药物治疗效果的评估[81]。在下面表中分别举例气管插管法在小鼠和大鼠(表 1)中，进行的处理方式，实验方法和研究目的。

Table 1. A model of *Pseudomonas aeruginosa* infection by intratracheal instillation
表 1. 气管插管法的铜绿假单胞菌感染模型

模型	麻醉剂	细菌量	方法	目的
小鼠				
急性感染	1%三溴乙醇	50 µl 含 $1\sim5 \times 10^6$ CFUs 琼脂珠悬浮液	1) 观察临床体征及监测死亡率； 2) 支气管肺泡灌洗； 3) HE 染色法。	1) 测定铜绿假单胞菌急性感染期间的毒力； 2) 检测细胞因子/趋化因子来分析宿主免疫应答； 3) 肺组织病理学分析。
慢性感染	氯胺酮(50 mg/ml)和赛拉津(5 mg/ml)	50 µl $1\sim2 \times 10^6$ CFU	1) 观察临床体征及监测死亡率； 2) 支气管肺泡灌洗； 3) HE 染色法。	1) 比较铜绿假单胞菌临床菌株与实验室菌株的毒力作用； 2) 检测细胞因子/趋化因子来分析宿主炎症反应； 3) 肺组织病理学分析。
大鼠				
急性感染	异氟醚	1×10^5 CFUs 琼脂珠悬浮液	1) 皮下注射 50 mg/kg 环丙沙星； 2) 支气管肺泡灌洗； 3) HE 染色。	1) 检测抗生素环丙沙星是否可以减轻肺部感染； 2) 检测细胞因子来分析宿主免疫应答； 3) 肺组织病理学分析。
慢性感染	七氟烷	150 µl 含 1×10^8 CFU/ml 琼脂珠悬浮液	1) 测定肺部细菌生长的时间进程及 HE 染色法； 2) 通过雾化角鲨胺(3 mg)进行治疗。	1) 评估肺部的细菌负荷情况和感染的严重程度； 2) 评估雾化吸入给药时对减少肺部细菌负荷的影响及评估角鲨胺的疗效。

气管插管法的优点在于：第一，能对感染的部位进行精确地控制给予的细菌量[81]。第二，可精确给予细菌感染于一侧肺中，不造成肺部以外产生感染作用[88]。此方法的缺点为，实验操作困难，手术麻醉及处理可能造成动物死亡[78]，手术造成的侵入性伤害及局部炎症反应也可能影响实验结果的解读[51]。

2) 鼻滴注

鼻滴注法是将细菌悬浮液经鼻孔滴注的方式给予，然后由动物吸入[74] [82] [83] [89] [90] [91] [92] [93]，主要实验动物多为小鼠(表 2)。鼻滴注法多用于急性肺部感染相关研究，对慢性肺部感染模型相关研究较少[83]。

Table 2. A mouse model of *Pseudomonas aeruginosa* infection by intranasal application
表 2. 鼻滴注法的铜绿假单胞菌感染小鼠模型

模型	麻醉剂	细菌量	方法	目的
急性感染	盐酸氯胺酮(65 mg/kg)和赛拉津(13 mg/kg)	20 μl 含 2×10^6 CFUs 细菌悬浮液	1) 测定组织中的细菌量； 2) 庆大霉素排斥试验； 3) 将 *exoU* 基因导入缺乏 *exoU* 基因的非细胞毒性铜绿假单胞菌菌株。	1) 表征小鼠的细菌感染过程； 2) 检测铜绿假单胞菌的内化； 3) 评估 *exoU* 基因对细菌毒力的影响。
慢性感染	氧气和异氟烷	50 μl 含 2×10^6 CFU/ml 细菌悬浮液	1) 感染后不同的时间段(0、6、12 h 和第 1、2、3、5、7、14、21 和 28 天)处死小鼠； 2) 第 1、3、7、14、21 和 28 天从每只小鼠鼻咽分离出铜绿假单胞菌； 3) 纸片扩散法。	1) 评估细菌数量和感染早期与生物膜形成和毒力相关的基因表达的变化； 2) 检测细菌表型变化； 3) 对所有分离株进行药敏试验。

相比于气管插管法，鼻滴注法侵入性较小，操作简单。然而，该方法滴入的菌液通过鼻、咽喉致部分菌液到达肺部，致使细菌剂量难以确定。且实验动物也可能发生上呼吸道感染[94]或其他以外的感染[82]。

3) 雾化吸入

雾化吸入法是由雾化器产生细菌气溶胶，送入于暴露室中，由动物将细菌气溶胶吸入肺中，达到细菌感染目的[84] [85] [95] [96]。雾化吸入方式广泛用于小鼠急性和慢性肺部感染研究中[84] [85] [97] (表 3)。

Table 3. A mouse model of *Pseudomonas aeruginosa* infection by aerosol
表 3. 雾化吸入法的铜绿假单胞菌感染小鼠模型

模型	麻醉剂	细菌量	方法	目的
急性感染	无	18 ml 低接种量 6.4×10^4 CFU 和高接种量 7.9×10^5 CFU	1) 支气管肺泡灌洗； 2) HE 染色法； 3) 微球阵列和 Luminex 100 分析。	1) 计算中性粒细胞； 2) 肺部组织病理学分析； 3) 分析 TLR5 对铜绿假单胞菌肺部感染的先天免疫反应的影响。
慢性感染	无	5 ml 含 1×10^{11} CFU/ml 细菌悬浮液	1) 使用 IL-10T 小鼠进行研究； 2) HE 染色法； 3) 肺组织匀浆中活菌平板计数； 4) 酶联免疫吸附。	1) 评估 IL-10 在与铜绿假单胞菌感染相关的炎症过程中的潜在作用； 2) 肺部组织病理学分析； 3) 监测铜绿假单胞菌的肺清除情况； 4) 检测细胞因子的表达。

雾化吸入法的优点在于动物无需身体约束，麻醉及手术侵入[82]。然而，该方法相较于气管插管差法和鼻滴注法成本较高，操作复杂，也不能精确地确定感染下呼吸道的细菌量，也容易发生上呼吸道感染或肺部以外的感染[51]。

3. 铜绿假单胞菌入侵机制

铜绿假单胞菌侵染呼吸道上皮细胞层的机制主要通过直接内化入侵上皮细胞或是经由解离细胞与细胞间的紧密连接达到入侵目的。为探究铜绿假单胞菌侵染呼吸道上皮细胞层的两种主要机制，实验可通过细胞模型或动物模型进行相关研究，且实验多集中于细胞模型上，而动物模型上研究较少[98]。下面将总结目前相关实验对铜绿假单胞菌入侵上皮细胞机制的研究进展。

3.1. 铜绿假单胞菌直接入侵上皮细胞

大多数铜绿假单胞菌内化的研究都是在细胞系中进行的。许多研究表明，呼吸道上皮细胞的极性影响铜绿假单胞菌感染的敏感性，因此极化较差的细胞或基底面暴露的细胞更容易受到铜绿假单胞菌的入侵和细胞毒性作用[99]。Katharine 等[100]为了探究铜绿假单胞菌进入上皮细胞的机制，通过用 GFP 标记的铜绿假单胞菌(PAO1)感染人的 CF 型支气管上皮细胞，研究发现铜绿假单胞菌可入侵 CF 型上皮细胞并进行复制，细菌在细胞中 24 h 内不产生细胞毒性，且突变的 CFTR 导致该细菌对支气管感染的易感性增加。朱鹏程等[101]研究通过用铜绿假单胞菌菌株 PAO1-EGFP 感染小鼠肺巨噬细胞，并静脉注射 CoB1，发现 CoB1 可通过泛素化介导的降解途径减少 PAK1 的表达，从而降低 Akt 的磷酸化水平，阻断 Akt/mTOR 信号通路，促进了 mTOR 释放 ULK1/2-Atg13-FIP200 复合体，启动自噬小体的形成，从而提高了宿主对细菌的清除能力。铜绿假单胞菌入侵上皮细胞后，可在上皮细胞内形成细菌的豆荚状集群，通过利用上皮细胞作为持续存在的避难所，并形成具有生物被膜生理学特征的可逆的抗生素耐药性表型，这可能有助于慢性感染的发展[98]。

3.2. 铜绿假单胞菌解离细胞间的紧密连接

紧密连接(Tight junction, TJ)是细胞－细胞界面上重要的蛋白质复合体，连接相邻细胞形成呼吸道上皮屏障，可防御病原微生物及其他物质入侵呼吸道上皮细胞[102]。铜绿假单胞菌感染的发病机制是复杂的，但通常情况下，破坏上皮细胞屏障是铜绿假单胞菌入侵上皮细胞的先决条件[103]。铜绿假单胞菌分泌的 III 型毒素(T3SS)是与临床感染相关的重要毒力因子，其中 Exo S 是 T3SS 效应器之一，已被发现可以破坏上皮紧密连接并促进细胞旁细菌的定位[104]。Exo S 其具有 N 端 RhoGTPase 激活蛋白(RhoGAP)结构域和 C 端 ADP 核糖基转移酶(ADPr)结构域的双功能酶，Exo S 的 RhoGAP 结构域可将 Rho、Rac 和 Cdc42 中的 GTP 水解为 GDP，导致细胞骨架解聚，从而降低宿主细胞吞噬铜绿假单胞菌的能力[105]。Exo S 通过损害紧密连接蛋白(包括闭合蛋白)对细菌渗透的防御功能，促进铜绿假单胞菌渗透到上皮屏障[106]。此外，Laurence 等[107]研究发现，分泌鼠李糖脂的铜绿假单胞菌菌株能有效地调节体外重组人呼吸道上皮中的屏障功能，而与该细菌释放弹性酶和脂多糖无关，且产生鼠李糖脂但具有 III 型分泌系统缺陷的菌株也渗透到上皮细胞，排除了 III 型机制是这种渗透的触发因素。刘金国等[108]为探讨空气中的颗粒物(PM)对人支气管上皮细胞经铜绿假单胞菌感染后的紧密连接的影响，通过共聚焦显微镜检测等实验发现 PM 可通过氧化应激破坏的上皮细胞间的紧密连接，促进铜绿假单胞菌入侵人支气管上皮细胞。

4. 总结

铜绿假单胞菌是一种致病病原体，可引起急性和慢性呼吸道感染。由于其具有很强的抗生素耐药性，且频繁的抗生素治疗会导致耐药菌的产生，所以治疗铜绿假单胞菌引起的呼吸道疾病变得尤为困难。由此，探究铜绿假单胞菌的致病机制及寻找潜在的治疗靶点变得至关重要。动物模型是体外试验和临床研究之间的关键一环。不同的动物模型对于细菌感染引起的呼吸道炎症反应的病理生理学研究、诊断和治疗具有重要意义。建立铜绿假单胞菌感染的动物模型对探究铜绿假单胞菌的致病机制及寻找潜在的治疗

靶点是必不可少的。然而，不同的细菌给予方式，对不同感染模型的结果有一定的影响。因此，在建立不同感染模型前，需要考虑模型的类型，麻醉剂的使用，测量指标和终点，操作简易程度，成本等因素，从而选择合适的细菌给予方式来建立感染的动物模型。

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