

噬菌体抗感染及临床应用进展

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【摘要】 近年来, 细菌耐药性问题愈发严重, 给全球公共卫生事业与医疗保健带来了困扰, 研发新型抗菌药物需要投入更多的时间与资金。噬菌体作为一类能够特异性感染细菌、真菌、放线菌等微生物的病毒, 可依赖宿主菌大量复制, 种类丰富, 且研发成本低, 在抗感染治疗方面的价值十分可观, 是具有巨大潜力的新一代生物抗菌剂。现通过对噬菌体杀菌机制、噬菌体抗感染方面研究进展以及临床应用进行综述, 以期对噬菌体抗感染治疗及临床应用提供参考。

【关键词】 噬菌体; 感染; 临床应用

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Anti-infection effect of phage and its clinical application

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【Abstract】 In recent years, the problem of bacterial resistance has become more and more serious, which has brought troubles to global public health and medical care. The time and money required to develop new antibiotics is even greater than before. Bacteriophage is a kind of virus that can specifically infect bacteria, fungi, actinomycetes and other microorganisms. Relying on host bacteria to replicate in large numbers, rich species, low research and development cost, the value of anti-infection therapy is very considerable. It is a new generation of biological antimicrobial agents with great potential. This paper briefly describes the sterilization mechanism, progress of research on anti-infection aspect and clinical application of phage, in order to provide reference for phage anti-infection treatment and clinical application.

【Key words】 Phage; Infection; Clinical application

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噬菌体最初由英国病理学家 Twort^[1] 在 1915 年发现。1917 年, D'Herelle^[2] 提出噬菌体概念, 并利用噬菌体悬浮液治疗痢疾, 为抗感染相关研究提供了新方向。20 世纪 30 年代后期, 当噬菌体疗法的安全性和疗效需大量研究验证时, 磺胺类药物和青霉素问世, 抗菌药物治疗的热潮逐渐削弱了研究者对噬菌体的热情^[3]。随着细菌耐药问题日趋严峻, 细菌对噬菌体广泛耐药情况较少的现象受到重视^[4-5], 使噬菌体疗法重新成为抗菌药物治疗的一种潜在的补充或替代疗法。现就噬菌体抗感染的研究进展及临床应用进行综述。

1 噬菌体杀菌机制

噬菌体是一类能够特异性感染细菌、真菌、放线菌等微生物的病毒, 既可以是 DNA 病毒, 也可以是 RNA 病毒, 其中大多数噬菌体是双链 DNA 基因组^[6]。不同类型噬菌体可以感染的细菌范围不同^[7], 这些差异由细菌表面的噬菌体受体所决定。根据噬菌体对宿主菌作用方式不同, 可将噬菌体分为毒性噬菌体及温和噬菌体。毒性噬菌体与特定的细胞表

面受体结合, 将自身遗传物质转入细胞内进行大量复制, 然后子代噬菌体通过裂解细胞来终止它们的感染, 释放到周围环境中的子代细胞结合周围细菌继续复制释放这一循环杀伤过程。在噬菌体的生命周期中, 裂解行为发生在毒性噬菌体的溶解周期及温和噬菌体发生溶源周期转变后^[8-9]。溶源周期是指噬菌体感染并将自身 DNA 整合到寄主细菌染色体 DNA 上, 在感染过程中没有产生子代噬菌体颗粒, 处于这种溶源周期的噬菌体是温和噬菌体。噬菌体进入溶源或溶原周期由 arbitrium 通讯系统决定。Arbitrium 是一种短肽信号分子, 它受到 aimP、aimR 和 aimX 3 个基因编码^[10]。

2 噬菌体制剂

2.1 早期噬菌体制剂: 最初研究者担心, 种类过多的噬菌体合成制剂在溶解细菌时, 细菌被裂解后快速释放的内毒素会引起局部甚至全身炎症反应, 噬菌体制剂大多以少量种类噬菌体混合为主, 这些混合制剂应用于活体组织较少发生不良反应, 但是限制了噬菌体杀灭菌种的范围, 临床上不能快速

确定菌株类型时,采用该方法治疗会缺乏及时性和针对性。

2.2 “鸡尾酒”疗法:“鸡尾酒”疗法即多种噬菌体混合而成的制剂,不仅扩大了噬菌体宿主范围,解决了单一噬菌体治疗病原菌的局限性,还避免了2种或以上生物膜抗噬菌体细菌的滋生^[11]。Wright等^[12]采用混合制剂成功治疗铜绿假单胞菌感染致慢性中耳炎。噬菌体混合制剂不是任意组合而成。在铜绿假单胞菌感染致肺囊性纤维化患者治疗中发现,由可识别不同靶细菌的噬菌体组成的混合制剂疗效更佳,这主要是因为识别相同靶细菌的噬菌体互相竞争,混合制剂中选择具备裂解不同菌株能力的噬菌体,在扩大靶细菌范围的同时增强了混合制剂活性^[13]。迄今为止已提出3种噬菌体混合物模型,即固定“鸡尾酒”配方、“鸡尾酒”库、非固定“鸡尾酒”配方^[14]。尽管已设计出适用于所有可能细菌靶标的噬菌体混合物,但噬菌体制剂的安全问题仍是一大挑战。

2.3 水解酶制剂:噬菌体对宿主菌的溶解依赖于噬菌体自身蛋白,其中噬菌体水解酶因为制备简易成为研究热点。发挥杀菌作用的噬菌体水解酶主要有两种,即外溶酶(在噬菌体生命周期的早期阶段促进基因组进入细菌)和内溶酶(在噬菌体生命周期的终末阶段降解宿主菌,使子代噬菌体释放),这两类酶可以破坏靶细菌生物膜^[15]。水解酶制剂能通过改造蛋白结构域拓宽杀菌谱。研究表明,抗鲍曼不动杆菌感染的噬菌体内溶酶 LysABP-05 的抗菌活性能被一种渗透肽-黏菌素增强^[16];治疗葡萄球菌感染的噬菌体肽聚糖水解酶 HydH5 与溶葡萄球菌酶的结构域 SH3b 的融合产物 HydH5SH3b 的裂解活性明显增加,融合产物能够裂解金黄色葡萄球菌和表皮葡萄球菌甚至耐甲氧西林的菌株^[17]。尽管水解酶制剂比噬菌体制剂研究更简易,但水解酶的耐药性和机体对酶的免疫应答反应仍然是需要解决的问题。

3 噬菌体与抗菌药物

噬菌体与抗菌药物同时作用于靶细菌的生物学作用远超过各自单独作用的总和,目前解释这一现象的机制主要包括:①噬菌体-抗菌药物协同作用:半数抑菌浓度的抗菌药物可以刺激靶细菌细胞壁的结构改变,引起噬菌体增加^[18]。虽然噬菌体与抗菌药物存在协同作用,但也出现与之矛盾的研究结果,因此还需进一步明确噬菌体与抗菌药物之间的作用关系。②消除生物膜:噬菌体可以破坏细胞外基质生物膜,使包裹其中的细菌暴露于抗菌药物,间接增强噬菌体的易感性^[19]。③噬菌体-抗菌药物“跷跷板”效应:在进化权衡原理基础上,菌株暴露于抗菌药物环境中,在基因选择下进化为耐药菌,进化后的菌株失去了耐噬菌体的特性;同理,暴露于噬菌体条件下的细菌在基因选择后失去了耐抗菌药物的特性^[20]。“跷跷板”效应机制在以下研究中得到验证:Ho等^[21]发现,细菌基因 *epaR* 的突变导致噬菌体对粪肠球菌的吸附减少,然而这一基因的改变导致细菌对达托霉素的敏感性增加,甚至还出现相反的现象,当抗菌药物诱导细菌的表型变化时,相应噬菌体对细菌的捕食能力增加。有趣的是,在金黄色葡萄球菌与β-内酰胺类、糖肽类、脂肽类抗菌药物之间也发生了“跷跷板”效应^[22]。

4 噬菌体制剂临床应用

当前,多重耐药菌感染是重症监护病房患者死亡的主要原因之一^[23],进入临床试验阶段的噬菌体制剂大多也针对多重耐药菌感染。多黏菌素曾在治疗革兰阴性菌重症感染中表现出强大的抗菌能力,但长期使用多黏菌素时细菌耐药情况仍然存在^[24]。噬菌体 PMK34 的溶菌酶 LysMK34 间接加强了多黏菌素对细菌的敏感性^[25-26]。针对临床常见多重耐药菌,现今的噬菌体制剂主要围绕铜绿假单胞菌、鲍曼不动杆菌、肺炎克雷伯菌和金黄色葡萄球菌进行研究^[27]。由乔治亚州 Eliava 研究所研制的化脓杆菌制剂以及俄罗斯 Microgen 生物技术公司研制的复杂化脓杆菌噬菌体制剂用于治疗假单胞菌相关尿路感染患者效果尚可,且未发生不良反应。Jault等^[28]对混合噬菌体治疗铜绿假单胞菌感染烧伤伤口的疗效和耐受性进行评估,该研究中混合噬菌体制剂 PP1131 是由12种天然抗铜绿假单胞菌的噬菌体混合制备而成,结果显示,与标准组相比,试验组患者很少发生脓毒症及脓毒性休克。Schooley等^[29]报道了1例68岁感染多重耐药鲍曼不动杆菌的糖尿病患者,使用多种抗菌药物治疗和经皮尿管引流假性囊肿后,患者病情在4个月内持续恶化,菌株活性试验筛选出9种具有裂解活性的噬菌体,将这些噬菌体经皮导管注入脓肿腔,同时使用抗菌药物协同治疗后,感染得到有效控制。Bao等^[30]报道了1例63岁复发性完全耐磺胺甲恶唑-甲氧苄啶肺炎克雷伯菌尿路感染女性患者,在使用噬菌体“鸡尾酒”疗法治疗后痊愈。Petrovic Fabijan等^[31]对13例严重金黄色葡萄球菌感染患者静脉注射噬菌体制剂 AB-SA01 作为辅助治疗,结果表明,AB-SA01 在严重金黄色葡萄球菌感染治疗中有效且无不良反应发生,验证了它在感染性心内膜炎和脓毒性休克治疗上的安全性。此外,广泛耐药的胞内寄菌,即结核分枝杆菌,也能通过改变噬菌体封装方式,如脂质性包裹噬菌体的封装方式,侵入哺乳动物的宿主细胞,再裂解宿主细胞内的靶细菌^[32]。另外,抗体库可采用噬菌体展示技术或其他组合方式构建。噬菌体是会感染细菌的病毒,利用噬菌体展示技术构建抗体库时,将大量序列插入噬菌体,其比例是使每个噬菌体克隆都生成单个抗体或抗体片段。研究者借助噬菌体展示技术可以调整抗体库的规模和多样性。利用噬菌体展示技术制备疫苗,无论在病毒、真菌甚至肿瘤治疗等领域均有其独特的优势^[33]。

5 总结与展望

总之,噬菌体的研发成本低且来源广泛,噬菌体和噬菌体蛋白均可以应用于抗感染,这些使噬菌体在多重耐药菌感染治疗上潜力巨大。目前尚未发现大剂量噬菌体应用于机体而引起的免疫不良反应^[34]。但是噬菌体的生物学特征仍需进一步研究,噬菌体的类型多样,其混合物的功效和耐受性的评估亦缺乏有力的证据支撑,为噬菌体的临床应用增加了不确定性。相信随着研究的不断深入,噬菌体作用机制将不断被揭示,噬菌体疗法的调控将不断被确定,噬菌体应用前景非常可观。

利益冲突 所有作者均声明不存在利益冲突

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