

青蒿素及其衍生物对口腔相关微生物的影响*

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【摘要】 口腔的适宜环境为微生物定植提供了条件,而口腔微生物是诱发口腔感染性疾病的重要原因,针对病原微生物的治疗是控制口腔感染性疾病的有效策略。青蒿素是从传统中医药黄花蒿中提取出的倍半萜内酯类化合物,由于其具高效低毒的抗疟效果而成为间日疟、恶性疟和抗氯喹疟疾治疗的首选药物。近年来,青蒿素已被证实具有抗细菌、真菌、病毒、其他寄生虫、肿瘤等效果,而部分微生物与口腔疾病密切相关,因此本文将对青蒿素及衍生物在口腔相关微生物方面的作用效果进行综述,总结分析之前的成果及进展,为深入研究提供参考,并展望新的研究方向。完善现有技术及相关标准以明确青蒿素及衍生物对效果存在争议的微生物的作用,扩大对口腔感染性疾病相关微生物的检测,在抗真菌领域明确与现有抗真菌药物的相互作用,这几个方向有待深入研究。另外在抗口腔感染性疾病研究过程中,青蒿素及衍生物的给药方案、潜在药物相互作用、毒副作用等方面是深入研究的必要条件,也是研究的新方向。随着制作工艺的成熟、相关研究的完善与口腔感染性疾病治疗潜在需求,青蒿素及衍生物在口腔微生物领域拥有广阔的发展前景,也为口腔相关药物的研发提供了新的契机。

【关键词】 青蒿素 青蒿素衍生物 口腔微生物 抗菌药 抗真菌药(中药)

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【Abstract】 The oral environment provides suitable conditions for the colonization of various microorganisms. However, the oral microbials could be the initial factors of some kinds of oral infectious diseases, therefore the treatment against oral microbial pathogens has become an effective strategy. Artemisinin, a kind of sesquiterpene lactone extracted from Traditional Chinese Medicine *Artemisia annua* L, is the first-line therapy to treat tertian malaria, subtertian malaria and anti-chloroquine malaria for its high efficiency and low toxicity. In recent years, artemisinin and its derivatives have also been proven to be effective against bacteria, fungi, viruses, parasites, and tumors, some of which are closely related to oral diseases. In this review, we summarize the potential effects of artemisinin and its derivatives on oral microorganism by analyzing previous research and latest progress to provide the evidence for further improvement, and look forward to the new research directions. Further studies are needed to improve existing technologies and standards to clarify the effects of artemisinin and its derivatives on microorganisms with controversial effects, to expand the detection of microorganisms associated with oral infectious diseases, and to clarify the interaction with existing antifungal agents in the field of antifungal diseases. In addition, in the study of anti-oral infectious diseases, artemisinin and its derivatives' administration scheme, potential drug interactions, toxic and side effects and other aspects are necessary conditions for further research, which is also a new direction of research. With the maturity of the production process, the improvement of relevant research and the potential demand for the treatment of oral infectious diseases, artemisinin and its derivatives have a broad prospect in the field of oral microorganisms, and provide a new opportunity for the research and development of oral drugs.

【Key words】 Artemisinin Artemisinin derivatives Oral microbes Anti-Bacterial agents
Antifungal agents (TCD)

口腔是连接人体内部环境与外部环境的通道,适宜的温度、湿度及营养供给为微生物定植生长提供了条件。口腔环境复杂而微生物种类也具有多样性,主要由细菌、真菌、病毒等组成,还包括原虫及支原体等其他微生物^[1-2]。通常情况下这些微生物不具致病性,但机体免

疫功能受损或宿主口腔微生态失衡时,其可引起多种口腔感染性疾病^[3],包括龋病、牙髓根尖周疾病、牙周疾病、口腔黏膜病等^[4-8],对口腔局部健康,甚至全身健康造成损害^[9-10]。因此口腔微生物与口腔感染性疾病的发生发展密切相关。

青蒿素(Artemisinin)是从传统中药黄花蒿(*Artemisia annua* L.)中提取的倍半萜内酯类化合物,通过分离提纯、化学合成及改性可得到二氢青蒿素、青蒿琥酯、蒿甲

* 国家自然科学基金(No. 81430011)和四川省科技计划项目(No. 2020YJ0227)资助

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醚、蒿乙醚等一系列衍生物^[11-12]。青蒿素凭借其起效快、疗效好、抗药少、毒性低等优势已成为一线抗疟药物^[13]。青蒿素衍生物也广泛适用于各类疟疾的治疗,其中双氢青蒿素、蒿甲醚尤其适用于治疗氯喹耐药的恶性疟及凶险型疟疾,而青蒿琥酯适用于脑型疟及各种危重疟疾的急救^[13-14]。青蒿素虽缺少其他抗疟药物具备的含氮杂环,但其内部特有的过氧化桥结构可断裂生成活性氧(reactive oxygen species, ROS)是发挥效果的关键^[15-18]。屠呦呦教授也凭借这一发现获得了2015年诺贝尔生理学及医学奖^[19-21]。近年来,青蒿素接连被发现具有抗细菌、抗真菌、抗病毒、抗其他寄生虫、抗肿瘤、抗炎及免疫调节等作用^[22-28]。其中金黄色葡萄球菌、大肠杆菌、变异链球菌、白色念珠菌等均与口腔感染性疾病密切相关^[22, 27-28]。目前,青蒿素及其衍生物在抗口腔微生物感染方面的研究进展迅速,主要集中于单独应用抗微生物及对现有抗微生物药协同增效等方向,近年来有诸多文献报道了青蒿素及衍生物在体外实验及动物实验中的抗微生物应用。因此,本综述对青蒿素及衍生物在口腔微生物方面的研究进行总结,并讨论青蒿素及衍生物在口腔感染性疾病方面的应用,以进一步开发青蒿素及其衍生物在口腔感染性疾病领域的应用,并深入研究其作用机制。

1 青蒿素及其衍生物与细菌

口腔中常见细菌包括链球菌、葡萄球菌、普雷沃菌、卟啉单胞菌、奈瑟菌、梭杆菌、乳杆菌、肠杆菌、螺旋体等^[29-31],主要引起的口腔感染性疾病包括龋病、牙髓根尖周病、牙周病、口腔黏膜病、口腔颌面部间隙感染及术后感染等^[4-8, 30-31]。近年来,青蒿素在抗菌领域的研究愈发广泛并被认为具有菌群调控作用^[32],但其抗菌性能始终未达成一致结论。部分学者发现青蒿素对金黄色葡萄球菌在内诸多微生物具有抗菌性^[22, 27],而也有学者提出截然相反的结论^[33]。

金黄色葡萄球菌是口腔常见的革兰阳性病原菌^[30],也是口腔颌面部间隙感染及颌面手术后感染的主要致病菌^[34-35],近年来研究发现口腔中金黄色葡萄球菌耐药株的检出加大了治疗难度^[36]。青蒿素类似物对金黄色葡萄球菌的最小抑菌浓度(minimal inhibitory concentration, MIC)约为0.09 mg/mL^[27],另有研究测定了青蒿素衍生物对金黄色葡萄球菌的抑菌环大小(inhibition zone diameter, IZD)> 1.8 cm^[37],以上均肯定了青蒿素的抗菌能力。有学者使用 β -环糊精装载青蒿素后发现其对甲氧西林耐药金黄色葡萄球菌(methicillin-resistant *Staphylococcus aureus*, MRSA)的抑制率高达99.94%,机制为细胞膜通透

性及呼吸作用的改变^[38],证明了青蒿素在抗耐药菌及载药领域良好的开发前景。在动物实验中,LI等^[39]发现青蒿琥酯对致死剂量热灭活金黄色葡萄球菌脓毒血症小鼠具保护作用,联合苯唑西林后可将小鼠死亡率降低至60%,机制为抑制Toll样受体2(Toll like receptor 2, TLR2)和核苷酸结合寡聚化结构2(Nod2)上调进而抑制细菌所造成的炎症反应。JIANG等^[40-41]通过相同动物模型进一步完善了青蒿琥酯协同苯唑西林抗MRSA菌株WHO-2的机制,除抗炎作用外青蒿琥酯可直接抑制WHO-2多重耐药转运蛋白(NorA, NorB, NorC)表达并提高抗生素在细菌胞内积累,以上动物实验证明了青蒿琥酯与现有抗菌剂的良好互作效果,提示了青蒿琥酯作为增效剂的良好应用价值。金黄色葡萄球菌是口腔颌面部感染的常见病原菌,而青蒿素及衍生物在体外实验^[27, 37-38]及动物研究^[39-41]中均对其表现出良好的作用效果,除直接的抑制作用外^[27, 37],还可与现有抗生素发挥协同效果^[39-41],作用机制包括直接影响细胞膜通透性与呼吸作用^[38],并促进抗生素在胞内积累^[40-41],并在抗耐药菌及载药领域有良好的前景^[38],为临床治疗口腔金黄色葡萄球菌感染提供了新的药物储备。

大肠杆菌是口腔颌面部手术后感染的革兰阴性主要致病菌^[31],在癌症患者化疗术后口腔中检出率明显升高^[41],是临床最常见的病原菌之一。近年口腔样本中超广谱耐药性大肠杆菌的出现提示现有抗菌药物的匮乏^[42],因此抗大肠杆菌感染及耐药的新药研发具重大意义。诸多学者通过体外实验证明了青蒿素类似物对大肠杆菌的抑菌效果(MIC= 0.09 mg/mL, IZD= 1.2 ~ 1.6 cm)^[27, 37]。然而在体内研究中青蒿素类的作用机制尚存在争议,WANG等^[43]发现青蒿素联合氨苄西林或舒巴坦可降低大肠杆菌脓毒血症小鼠死亡率,但青蒿素并未直接抗大肠杆菌,而是抑制细菌介导的部分炎症反应,如核因子 κ B、肿瘤坏死因子 α 及白细胞介素6等炎性因子释放,且并未抑制TLR4、TLR9上调;而LI等使用青蒿琥酯在相同的动物模型得到截然相反的机制,通过测定分级抑制浓度系数(fractional inhibitory concentration index, FICI< 0.5)及正定霉素在胞内的积累后,发现青蒿琥酯可对 β 内酰胺类抗生素协同增效,直接抑制大肠杆菌的主要药物外排泵多药耐药系统AcrAB-TolC,进而增加抗生素在其胞内积聚,此外TLR-4与TLR-9的转录水平下调^[43-45]。由此可见,青蒿素类化合物对大肠杆菌具有体外抗菌活性^[22, 27, 37],然而动物实验中除了抗炎作用外^[43-45]是否对大肠杆菌有直接作用依旧有待研究^[44-45]。此外,青蒿素与青蒿琥酯虽同属倍半萜烯内酯类,但作用效果及机制可能存在差异^[43-45],

在临床应用及实验室研究中应谨慎处理。另有团队设计合成了若干针对大肠杆菌的青蒿素衍生物抗菌增效剂并表现出良好效果^[46-47],提示了这类化合物在抗菌增效方面的潜在开发价值。

变异链球菌为龋病发生发展的关键微生物,可通过分解有机物产酸导致牙体硬组织破坏^[3],研究表明青蒿素衍生物对变异链球菌具抑菌效果($IZD=1.2\sim 1.6\text{ cm}$)^[37],提示了其在抗龋方面的应用价值。除变异链球菌外,幽门螺杆菌凭借细胞结构的特殊性及其抗药性受到广泛关注。口腔中的幽门螺杆菌可影响牙菌斑的形成、牙周组织健康、口腔癌及干槽症的发生发展,并与胃部幽门螺杆菌感染密切相关^[48-49]。研究表明多种青蒿素衍生物均可影响幽门螺杆菌的生长,青蒿醚可有效抑制敏感及耐药幽门螺杆菌生长($MIC=0.25\sim 1.0\text{ mg/L}$),并在胃酸pH下药效稳定^[37];双氢青蒿素对胞内幽门螺杆菌具清除效果^[50];青蒿酮与阿莫西林($FICI=0.515$)及克拉霉素($FICI=0.53$)具协同抗幽门螺杆菌潜在价值^[50]。以上研究证明了青蒿素抗幽门螺杆菌的潜力,但是相关研究缺乏机制探索。此外,青蒿素在酸性环境下的稳定性及对变异链球菌的抗菌效果为其抗龋应用提供了可行性^[37]。

青蒿素及其衍生物对诸多口腔细菌具有抗菌能力,其效果包括直接抑菌作用、间接抗菌增效及抗细菌介导的炎症反应。其机制主要涉及细胞膜通透性改变^[38, 51],影响细胞呼吸^[36],促进其他抗菌药物在胞内积聚^[40-45]。此外,青蒿素衍生物在酸性条件下的稳定性与对变异链球菌的抗菌效果^[37],对牙周致病菌,如放线共生放线杆菌、中间普雷沃菌、具核梭杆菌存在一定抗菌能力^[52],进一步提示了其在牙体牙周疾病及口腔颌面部感染治疗中的潜在应用价值。

2 青蒿素及其衍生物与真菌

真菌是真核细胞型微生物,结构较细菌更为复杂。口腔中最常见的机会性致病真菌是白色念珠菌,在正常人口腔检出率约50%^[53],所引起的口腔念珠菌病可占总数的80%^[54],并与龋病、根尖周病、牙周病的发生发展密切相关^[55-57]。由于抗真菌药物更新缓慢,白色念珠菌耐药始终是有待解决的难题^[58],而青蒿素由于其广阔的作用效果及潜在的抗菌性能也在抗真菌领域受到了关注。

早期研究认为青蒿素对白色念珠菌作用效果微弱^[59],而近年诸多研究均证实了青蒿素在抗白色念珠菌感染方面的价值。研究表明青蒿素单独作用下缺乏抗白色念珠菌效果,对白色念珠菌生长无明显抑制作用($MIC>50\text{ }\mu\text{g/mL}$)^[59],即使提高药物浓度依旧无法单独抗白色念

珠菌($MIC>120\text{ }\mu\text{g/mL}$)^[60]。然而进一步研究却发现青蒿素具有抗真菌增效的潜能,青蒿素提高了酮康唑抗真菌效果,机制与多效性药物转运蛋白(PDR)介导的转运竞争有关^[60]。此后多位学者分别研究了青蒿素与不同抗真菌药物的相互作用。有学者提出青蒿琥酯对氟康唑具抗白色念珠菌增效能力,联合处理可使真菌生长较氟康唑单药对照组降低至67%^[61];另有学者否定了二者的协同效果,由于其FICI值未能达到协同标准($FICI>0.5$)^[62]。多种青蒿素衍生物均可与咪康唑在抗白色念珠菌生物膜时表现出良好的协同能力($FICI=0.069\sim 0.171$),该过程中ROS未发生变化^[62],提示协同机制并非过氧化物杀伤作用^[17-18]。两性霉素B是经典抗真菌剂,在抗恶性疟原虫研究中青蒿素与热灭活两性霉素B表现出良好协同能力^[63],然而其衍生物青蒿琥酯与两性霉素B并未在抗白色念珠菌时表现出协同效果($FICI>0.5$)^[62],说明同一药物组合对不同微生物的效果存在差异。综上,青蒿素及衍生物单独抗白色念珠菌效果微弱^[59-60],却可与部分现有抗真菌药物发挥协同效果^[60-62],其作用机制推测与药物竞争性外排相关^[60],而ROS在这一过程中未发生改变^[62],提示青蒿素在抗白色念珠菌领域可能存在新作用靶点,深入研究青蒿素与现有抗真菌药物的相互作用有助于发现青蒿素的新靶点,并可对临床治疗白色念珠菌感染提供指导。

酿酒酵母是最典型的真核生物模型,在遗传学上与白色念珠菌密切相关,常伴饮食在口腔及消化道中定植^[64-65]。诸多学者均通过酿酒酵母深入分析了青蒿素的作用机制。MOORE等^[66]分析了青蒿素对酿酒酵母线粒体膜去极化、ROS生成、钙通道编码基因的影响,认为青蒿素具两种生化活性:特异性靶向 Ca^{2+} -ATP酶;非特异性影响线粒体功能及自由基生成。针对 Ca^{2+} -ATP酶这一机制,有研究比较了不同生长环境下青蒿素对酿酒酵母 Ca^{2+} -ATP酶缺失株的作用,发现青蒿素可抑制内质网 Ca^{2+} -ATP泵从而抑制酵母生长^[67]。针对酿酒酵母线粒体功能这一机制,有研究证明线粒体在青蒿素作用过程中发挥双重作用:电子传递链活化青蒿素,而线粒体随后被活化后局部生成的自由基破坏^[68],而超氧化物歧化酶(superoxide dismutase, SOD1)可恢复ROS的生理水平以及线粒体膜电位^[69]。进一步研究认为青蒿素主要通过非特异性产生ROS及特异性抗线粒体两种途径抗酵母菌^[70],青蒿素对线粒体功能的影响主要通过3种作用机制:血红素介导的ROS产生;一般浓度下抗线粒体活性,无法被SOD1抑制;高浓度下抗线粒体活性,可被SOD1抑制^[71]。酿酒酵母的研究中揭示了青蒿素的多种作用机制,为青蒿素抗口腔真菌感染提供了理论基础。

青蒿素具微弱抗真菌能力^[59-60],却可与多种抗真菌药物相互作用^[60-63],青蒿素的抗真菌机制涉及药物竞争外排^[60]、Ca²⁺-ATP酶^[66-67]、ROS合成及线粒体功能^[67-71]等。除以上机制,青蒿素也存在其他潜在抗真菌靶点如赖氨酸脱乙酰酶^[72]。青蒿素对酿酒酵母的作用机制虽为抗白色念珠菌提供了研究思路,而抗真菌效果的差异进一步提示了二者可能存在不同的作用通路,有待进一步明确。

3 青蒿素及其衍生物与原虫

口腔中原虫微生物主要为牙龈内阿米巴和口腔毛滴虫,主要定植于龈沟内,已有研究报道青蒿素具抗阿米巴及抗毛滴虫能力。牙龈内阿米巴是口腔前庭内最常见的原虫,在正常口腔中检出率可达15%,而在牙周炎症区域内检出率可高达77%^[73],牙龈内阿米巴可定植于牙周袋内并侵入上皮组织,与牙周炎发生发展密切相关^[74-75]。研究表明青蒿素及双氢青蒿素对阿米巴原虫具有杀伤作用(50% inhibiting concentration, IC₅₀=18 μmol/L、9 μmol/L)^[76];高浓度蒿甲醚甚至可杀灭阿米巴滋养体(200 μg/mL),可作为磷酸甘油酸脱氢酶抑制剂在丝氨酸生物合成途径中阻断阿米巴感染^[77]。

口腔毛滴虫被认为与牙龈炎、牙周炎、龋齿及冠周炎有关^[78-79],研究表明青蒿素也具有抗毛滴虫作用。青蒿素对甲硝唑耐药和敏感毛滴虫均表现出杀灭活性(IC₅₀=280~560 μmol/L)^[80],进一步观察双氢青蒿素(1 mg/mL)对毛滴虫的作用机制,发现双氢青蒿素处理后的毛滴虫体胞膜变形、破裂,内容物外漏,虫体裂解,提示双氢青蒿素对毛滴虫膜系结构的破坏作用^[81]。以上研究表明青蒿素作为一线抗疟药物,有防治口腔原虫感染的潜在价值,对威胁牙周组织健康的阿米巴目及毛滴虫目原虫存在抑制效果^[76-77, 80-81],并且该效果对甲硝唑耐药原虫有效^[80],提示其独特的作用机制,除类似抗疟作用中膜系结构的破坏外^[81],还可能涉及丝氨酸合成通路的改变^[77],提示其潜在靶点及在口腔原虫感染治疗研究中广泛的应用前景。

4 青蒿素及其衍生物与病毒

口腔病毒感染与口腔黏膜疾病的发生密切相关。其中,疱疹病毒在口腔的检出率最高,可导致口腔黏膜疾病、牙周疾病及三叉神经痛等,极大影响患者的口腔健康^[82-84]。青蒿素及其衍生物被发现对双链DNA疱疹病毒具有很强的抑制作用,包括1型单纯疱疹病毒(herpes simplex virus, HSV-1)、巨细胞病毒(cytomegalovirus, CMV)、人疱疹病毒6(human herpesviruses, HHV-6)和EB病毒(epstein-barr virus, EBV)^[17, 85]。

青蒿琥酯对HSV具有体外抑制能力(0~30 μmol/L),并且与伐昔洛韦联合治疗HSV脑炎过程中可明显提高小鼠生存率^[86];同样,青蒿琥酯(30 μmol/L)能显著抑制CMV复制,活性与视网膜母细胞瘤蛋白磷酸化高度负相关,细胞周期蛋白依赖性激酶(cyclin-dependent kinases, CDK2)核抑制剂可抑制其磷酸化进而提高青蒿琥酯抗病毒活性^[87];此外,青蒿琥酯还可通过抑制病毒基因复制与早晚期蛋白合成而发挥抑制HHV-6的活性[IC₅₀=(3.80±1.06) μmol/L]^[88];青蒿琥酯对EBV也具有抑制作用[IC₅₀=(6.4±2.7) μmol/L],并可以降低病毒在细胞内的复制,其机制为影响细胞周期早期蛋白的合成^[89]。综上,青蒿琥酯表现出明显的抗疱疹病毒能力,其浓度多在微摩尔级别^[87-89],作用机制主要影响细胞周期,包括基因复制和蛋白合成^[87],尤其是早期蛋白合成^[88-89],CDK核抑制剂的增效能力也支持这一结论^[87]。以上研究不仅体现了青蒿素衍生物广泛的作用范围,更明确了该类药物抗口腔病毒感染的应用前景,具有良好的开发价值。

5 总结

青蒿素的发现是我国中医药发展史上的里程碑,随着相关研究的增多,青蒿素及其衍生物在微生物领域有非常广泛的研究范围,对口腔细菌、真菌、原虫、病毒感染防治均有良好效果。近年来,青蒿素类药物的生物合成及提取工艺也日渐成熟,为其广泛应用奠定了基础^[90]。然而,大部分研究依旧局限于体外抗微生物性能分析,部分作用效果尚无定论,而其作用机制研究也较为匮乏。因此,本团队认为以下方向有待深入研究:完善现有技术及标准以明确青蒿素及衍生物对效果存在争议的微生物的作用,如金黄色葡萄球菌等;扩大对口腔感染性疾病相关微生物的检测,如梭杆菌属、普雷沃菌属、卟啉单胞菌属、放线菌属、螺旋体及非白色念珠菌致病真菌;在抗真菌领域明确与现有抗真菌药物的相互作用,如氟康唑、两性霉素B并深入研究其作用机制。另外在抗口腔感染性疾病研究过程中,青蒿素及衍生物的给药方案、潜在药物相互作用、毒副作用等方面是深入研究的必要条件,也是研究的新方向。即便如此,随着制作工艺的成熟、相关研究的完善与口腔感染性疾病治疗潜在需求,青蒿素及衍生物在口腔微生物领域拥有广阔的发展前景,同时,也为口腔相关药物的研发提供了新的契机。

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(2020-07-06收稿, 2020-10-11修回)

编辑 汤洁