科学通报 2017 年 第 62 卷 第 18 期: 1928 ~ 1937
专辑: 青蒿素研究与发展 评 述
《中国科学》杂志社
SCIENCE CHINA PRESS

青蒿素与疟疾: 药物激活、作用机理及抗药性的研究进展

苏新专[1,2*], 李剑[2]

1. Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda 20892, USA;
2. 厦门大学生命科学学院, 细胞应急生物学国家重点实验室, 细胞信号网络协同创新中心, 厦门 361005

* 联系人, E-mail: xsu@niaid.nih.gov

2017-02-14 收稿, 2017-03-01 修回, 2017-03-02 接受, 2017-04-05 网络版发表
国家自然科学基金(81220108019, 81572017)、国家外国专家局和教育部"111 计划"(B06016)和美国国家过敏和传染病研究所项目资助

摘要 如能及早确诊并合理救治, 疟疾是可以通过抗疟药来治愈的. 然而, 抗药性疟原虫通常在一种新型药物的大规模使用后的数年内就会出现. 随着疟原虫几乎对所有类型的抗疟药均产生了不同程度的抗性, 青蒿素类药物联合疗法(ACTs)也由此成为治疗疟疾最重要的手段. 不幸的是, 已有报道称在东南亚地区采用青蒿素或ACTs治疗后出现了延迟原虫清除的现象, 这也让研究者们对青蒿素及其衍生物治疗在未来发生完全失效的可能感到担忧. 本文简要综述了青蒿素的药物激活、作用机理、药物靶点及可能的抗药性机制等研究的进展; 对抗药性的定义、青蒿素组合用药中伴侣药物的选择, 以及当前为消除疟疾采取全民用药的努力等问题作了讨论. 与此相关的议题已有大量的研究和文献报道, 由于篇幅有限未能逐一列举. 此外, 本文所讨论的某些问题仍存争议、还需深入的研究方能解答.

关键词 青蒿素, 疟原虫, 组合药物, 全民用药, 青蒿素类药物联合疗法

1 疟疾与疟原虫

疟疾是一种致命的疾病. 在2015年, 全球有2.14亿人感染疟疾, 约有35万人因疟疾死亡[1]. 引起人患疟疾的疟原虫有5种, 包括恶性疟原虫(*Plasmodium falciparum*)、间日疟原虫(*P. vivax*)、卵形疟原虫(*P. ovale*)、三日疟原虫(*P. malariae*)和诺氏疟原虫(*P. knowlesi*)[2]. 其中恶性疟原虫是造成患者致死率最高的疟原虫, 而间日疟原虫流行最为广泛. 疟原虫具有一个复杂的生活史. 首先, 雌性按蚊叮咬人体吸血时子孢子随唾液进入血流, 数分钟内子孢子侵入肝细胞、在细胞内逐渐发育并进行裂体生殖, 裂殖体发育成熟后胀破所寄生的肝细胞并释放出成千上万个裂殖子随即进入血流. 裂殖子侵入血红细胞进行红内期的无性裂殖循环、释放出更多的寄生虫, 完成一代红内期无性生殖一般需要2~3天的时间. 伴随寄生红细胞的破裂、虫体和宿主细胞内容物的释放, 将引起患者产生发烧、寒颤、冒汗、反胃、呕吐及身体疼痛等疟疾症状. 有些感染是极其严重和致命的, 导致诸如脑型疟、重症贫血、低血糖症、呼吸窘迫综合征(acute respiratory distress syndrome, ARDS)、急性肾衰竭和重度原虫症等[2,3]. 在红内期, 部分裂殖子侵入红细胞后不再进行无性生殖转而进行有性发育成雄配子体和雌配子体, 这种有性生殖转变的具体机制目前尚不清楚. 当按蚊叮咬患者吸血时, 雌、雄配

引用格式: 苏新专, 李剑. 青蒿素与疟疾: 药物激活、作用机理及抗药性的研究进展. 科学通报, 2017, 62: 1928–1937
Su X Z, Li J. Artemisinin and malaria: Current understandings of drug activation, action, and resistance (in Chinese). Chin Sci Bull, 2017, 62: 1928–1937, doi: 10.1360/N972017-00147

子体随血流进入蚊体内继续发育成雌、雄配子; 雌配子和雄配子受精发育形成具有运动能力的动合子. 动合子穿过蚊胃壁形成卵囊, 卵囊进行孢子增殖生成成千上万的子孢子. 子孢子随血淋巴迁移至蚊唾液腺, 受感染的按蚊再次叮咬人体吸血, 子孢子便可重新侵入新的宿主. 红内期原虫, 尤其是处于活跃生长阶段的滋养体和裂殖体对抗疟药较为敏感, 这也是药物治疗的主要攻虫时期.

2 抗疟药和抗药性

尽管目前缺乏有效的疟疾疫苗, 但如果患疟病人能被及早确诊和合理治疗, 是可以通过使用不同的抗疟药来治愈的. 抗疟药的种类繁多, 包括4-氨基喹啉(如氯喹 (chloroquine, CQ)、阿莫地喹(amodiaquine, AMQ)和哌喹(piperaquine, PPQ))、8-氨基喹啉(如伯氨喹 (primaquine, PMQ)) 、芳胺基醇(如甲氟喹(mefloquine, MQ)、本芴醇(lumefantrine, LUM)和奎宁(quinine, QN))、抗叶酸类(如乙胺嘧啶(pyrimethamine, PYR)和磺胺多辛(sulfadoxine, SDX))、过氧基倍半萜内酯(青蒿素及其衍生物)及其他类型的药物(咯萘啶(pyronaridine, PND)、阿托伐醌(atovaquone, ATQ)、氯胍(proguanil, PGN)、多西环素(doxycycline, DXC)和克林霉素(clindamycin, CDM))[4,5]. 其中氯喹、乙胺嘧啶-磺胺多辛曾经是行之有效且价格低廉的药物, 不幸的是, 由于在疟疾流行区出现了与其相对应的抗药性疟原虫, 这些药物也随之被弃用. 除奎宁和青蒿素之外的其他抗疟药均在引入临床治疗后的数年内就产生了抗药性, 甚至, 有些药物如阿托伐醌和乙胺嘧啶从引入到抗药性出现的时间还不足一年[6]. 抗药性也成为影响和阻碍疟疾防控与根除的重要问题. 由于疟原虫普遍地对氯喹、乙胺嘧啶和磺胺多辛等药物产生抗性, 世界卫生组织(World Health Organization, WHO)于2005年建议以青蒿素类药物联合疗法(artemisinin combination therapy, ACTs)取代其他的治疗手段[7,8]. 当前, ACTs已在世界范围内广泛使用并挽救了数以百万计人的生命. 然而, 已有报道称在东南亚地区采用青蒿素或ACTs治疗后出现了延迟原虫清除(delay parasite clearance, DPC)的现象[9~11], 因此必须时刻警惕青蒿素及其衍生物治疗在未来发生完全失效的可能. 使用标准的72 h体外测药试验, 氯喹抗性虫株, 以及乙胺嘧啶-磺胺多辛抗性虫株比敏感性虫株的IC_{50}(药物的半抑制浓度)普遍高出10~1000倍. 幸运的是, 迄今为止从野外采集的所谓的青蒿素抗性虫株其IC_{50}并没有显著高于敏感性虫株. 事实上, 从青蒿素单方引入临床治疗后, DPC就一度被定义为青蒿素抗性. 这种治疗后延迟原虫清除的现象主要发现在东南亚地区如泰国-柬埔寨边境[9,10]. 在学术界, DPC是否可以认为是出现了真正的抗青蒿素类药物的虫株、乃至与此相关的青蒿素抗性的定义还存在很多争议.

为了更好地理解疟原虫抗药性, 首先需解读疟疾治疗时的耐药性. 根据WHO对疟原虫耐药性的定义, 传统上将寄生虫对一种药物的反应分为4种类型(分别是S, RⅠ, RⅡ和RⅢ)[12]: 敏感性S定义为首次用药后的7天内无性期原虫被清除且无复发; 抗性RⅠ定义为用药后无性期原虫被清除但有复发; 抗性RⅡ定义为用药后能显著降低无性期原虫率但不能完全清除原虫; 抗性RⅢ定义为用药后不能显著降低无性期原虫率. RⅡ和RⅢ类型的氯喹抗性、乙胺嘧啶-磺胺多辛抗性及其他药物抗性已有大量的报道. 属于RⅡ和RⅢ类型的寄生虫其提升的药物半抑制浓度(the half maximal inhibitory concentration, IC_{50})通常可以采用体外的药物抑制原虫生长试验来进行测定. 通过体外测定IC_{50}和体内的测药实验证实, 恶性疟原虫的氯喹抗性和乙胺嘧啶抗性分别是由于氯喹抗性转运蛋白编码基因(*pfcrt*)和二氢叶酸还原酶编码基因(*pfdhfr*)的突变引起的[13,14]. 抗药基因的突变可导致IC_{50}的值增加数十甚至数千倍. 通常这些基因的突变位点也被用作群体抗药性筛查的分子标记[15]. 而青蒿素抗性的测验却是另外一番景象. 根据标准的青蒿素类药物的DPC测试方案(每天用药剂量2~4 mg kg^{-1}体重, 用药3天), 延迟半数原虫清除时间(PC1/2)>5 h和有K13基因突变型(在后面有详细讨论)定义为青蒿素抗性虫株[16,17]. 然而, 报道的PC1/2>5 h的虫株和PC1/2<3 h的虫株它们之间的IC_{50}值并没有多大差别. 这是由于PC1/2会受到诸如宿主免疫力、发热、贫血以及原虫密度等因素的影响, 这些因素可能造成PC1/2值多至2倍的差异[16,17], 因此采集患者血液直接测试很难对PC1/2表型进行精确测定. 为解决这一问题, 研究人员建立了一种名为环状体生存试验(ring survival assay, RSA)的体外测试方法, 该方法是基于在青蒿素短时间处理后有些虫株的环状体具有较高存活率的发现[18], 结果显示RSA值与DPC值之间有很好相关性. 恶性疟原虫其RSA测试的存活率

≥1%被定义为青蒿素抗性[19]. PC1/2和RSA也成为测试一株疟原虫是否具有“青蒿素抗性”的标准[20]. 疟原虫对青蒿素耐受是否增加的另一个指标是观察治疗后复发率是否升高. 然而, 在早期的青蒿素临床治疗就有发现很高的治疗后复发的现象[21], 很难判断这种再次发作是由于复发还是二次感染造成的[6]. 因此, 这种青蒿素抗性的表型更像是之前WHO定义的RⅠ抗药类型. 为克服青蒿素在体内的半衰期短而导致的高复发率问题, 通常需要使用另外一种半衰期长的药物与青蒿素组成复方以提高治愈率[21]. 遗憾的是, 在柬埔寨西部ACTs的治疗效果也在降低, 例如, 双氢青蒿素-哌喹复方(DHA/PPQ)的治愈率从10~15年前的~98%下降到目前的75%~89%[11,22~24]. 治疗效果的降低很可能是由于疟原虫对复方的伴侣药物哌喹的敏感性减小了[17].

概括来说, 尽管在东南亚地区青蒿素类药物治疗后有延迟原虫清除和复发率升高的报道, 青蒿素类药物及其复方仍能有效地杀灭疟原虫. 目前还没有报道或证据证实产生了RⅡ和RⅢ类型的青蒿素抗性. 虽然最近有报道源于非洲的一株带有K13突变(M579I)和DPC的抗性寄生虫[25], 标准的ACTs在非洲疟疾流行区仍然有效, 其治愈率高且极少出现延迟原虫清除的情况.

3 青蒿素的激活和潜在作用靶点

青蒿素是一种含有特殊过氧桥基团的倍半萜内酯, 这种过氧基团被认为与青蒿素的药物活性有关. 通过内过氧桥的断裂产生强反应性自由基可能是青蒿素起杀虫作用的关键[26], 游离的亚铁离子和亚铁血红素被认为是激活青蒿素的主要元素[27~31]. 早期环状体其生物合成途径产生的血红素和发育晚期中消化血红蛋白产生的血红素均是青蒿素的活化剂[31]. 在一项研究中, 发现使用半胱氨酸蛋白酶抑制剂抑制血红蛋白酶的活性、敲除半胱氨酸蛋白酶falcipain-2或直接去除宿主细胞的裂解产物都能显著降低寄生虫对青蒿素的敏感性, 这暗示青蒿素的激活有赖于血红蛋白的消化水解及此过程中释放的血红素. 研究也发现, 青蒿素存在的情况下寄生虫会减少摄取血红蛋白[30]. 最近, Wang等人[31]通过体外结合试验证明青蒿素的主要激活剂是亚铁血红素而不是游离的亚铁离子. 根据研究人员提出的模型, 青蒿素的激活依赖于疟原虫在生物合成途径中和消化血红蛋白中产生的血红素. 在早期环状体时期, 疟原虫体内合成的血红素是激活青蒿素的主要来源; 而在疟原虫发育晚期, 消化血红蛋白产生的和生物合成途径产生的血红素对青蒿素的激活均有贡献[31]. 由于早期环状体尚未开始大量消化血红蛋白, 因此可能无法有效地激活青蒿素, 所以此阶段对青蒿素有较高的耐性; 而处于活跃消化血红蛋白状态下的滋养体对青蒿素更为敏感. 这就解释了为什么有些环状体在青蒿素短暂处理后能存活(存活时间可达6 h), 这也应该是RSA的实验基础.

下一个问题是: 青蒿素的药物靶点是什么, 或者说激活的青蒿素是如何杀死寄生虫的? 使用非洲爪蟾(*Xenopus laevis*)卵母细胞表达系统, 有报道称青蒿素能显著抑制恶性疟原虫ATP酶6(PfATP6或SERCA)的活性, 且具有与毒胡萝卜素(也属于倍半萜内酯, 是SERCA高度特异的抑制剂)相似的功效[27]. 由于PfATP6是SERCA型的钙离子ATP酶, 用螯合剂去铁离子也可抑制青蒿素的活性. 然而, 后续的一些研究未能证实PfATP6是青蒿素的药物靶标, 酵母异源表达纯化获得的具酶活性的PfATP6不能结合青蒿素或抑制其活性[32]. 有研究也调查了在青蒿素临床治疗前后PfATP6编码基因的突变点, 但未观察到明显的关联性[33,34]. 这些研究不大可能获得有意义的信息, 因为还没有RⅡ或RⅢ型青蒿素抗性的报道. 后来, 首次报道PfATP6是青蒿素靶点的研究组又通过在酵母中表达PfATP6, 发现青蒿素有抑制PfATP6的活性. 该项研究中, 从野外分离株(S769N)和实验室克隆株(L263E)获得的突变型PfATP6在酵母中表达时均能降低对青蒿素的易感性, 但对其他无关的抑制剂如环唑酸的易感性增加了[35]. 有趣的是, 寄生虫对青蒿素及其衍生物的反应和对许多钙离子通道抑制剂的反应有高度相关性, 表明寄生虫对青蒿素和钙离子代谢或转运有着重叠的机制[36,37]. PfATP6在寄生虫对青蒿素的应答中所起的作用仍需进一步研究.

最近的一项研究显示, 恶性疟原虫的磷脂酰肌醇-3-激酶(phosphatidylinositol-3-kinase, PfPI3K)也可能是青蒿素的作用靶点[38]. 该研究发现, PfPI3K的脂质产物磷脂酰肌醇-3-磷酸(phosphatidylinositol-3-phosphate, PI3P)水平的升高可作为临床上和实验室筛选的青蒿素抗性寄生虫的一个指标. 最近报道的疟原虫Kelch13编码基因(*PfKelch13*)的突变可造成PfPI3K与PfKelch13蛋白结合的减少并降低PI3K的多

聚泛素化, 最终导致PfPI3K蛋白水解的减少, 从而增加PI3P的水平[38]. 由于这是一个相对较新的发现, 此结果仍需深入研究才能确定.

如果反应性自由基是杀死寄生虫的因子的假说成立, 则青蒿素的靶点可能有多个. 事实上, 使用活性探针标记青蒿素和蛋白质组学分析, Wang等人[31]发现, 124个疟原虫蛋白可结合青蒿素, 其中许多蛋白参与寄生虫的多个重要的生物代谢途径, 如羧酸代谢、细胞生物胺和核苷以及核糖核苷的生物合成等. 在钙离子转运系统或代谢途径中起作用的多个分子, 包括钙离子结合蛋白、钙依赖性蛋白激酶和两种钙离子ATP酶通道蛋白(ATP4和ATP6), 被推测是青蒿素的可能作用靶点. 使用体外蛋白结合试验, 他们还验证了之前鉴定的青蒿素靶标——恶性疟原虫翻译调控肿瘤蛋白(translational controlled tumor protein, TCTP)同源物, 此蛋白被认为可结合钙离子并具有稳定微管的作用[39]. 这些发现支持了PfATP6及参与钙离子代谢的分子对青蒿素的应答有一定的作用. 有趣的是, PfMDR1和PfCRT也在他们确定的靶标中, 预示着这两个分子也在青蒿素的运输和代谢中起作用. 另一个与之相似的研究, Ismail等人[40]也鉴定了许多参与糖酵解、血红蛋白降解、抗氧化防御和蛋白质合成途径的可能作用靶点. 同样, PfCRT, PfMDR1和PfATP6 (PF3D7_0106300, 钙离子ATP酶通道蛋白)也被鉴定是青蒿素的靶点. *pfcrt*编码序列的突变[41,42]和*pfmdr1*编码序列的突变或基因表达量的变化[43,44], 均能改变疟原虫对青蒿素的半致死量IC_{50}浓度, 尽管这个改变相对较小. 这些结果与最近的有关青蒿素和钙/钠离子通道抑制剂共享转运途径或作用机制, 以及PfMDR1和PfCRT可以调节它们间相互作用的报道是一致的[37]. 在一项使用HeLa细胞的蛋白组学研究中, Zhou等人[45]也鉴定了79种青蒿素-烷基化蛋白, 包括两种已知的青蒿素靶标TCTP (fortilin)和SERCA2. 这些研究结果都表明PfCRT, PfMDR1及钙离子转运相关的分子如PfATP6在青蒿素的转运或代谢中起作用. 如果在将来不幸产生RⅡ/RⅢ型的青蒿素抗性, 那么主要的抗药性候选基因将可能是这3个基因中的一个或多个. 有报道称青蒿素类药物能干扰疟原虫对血红蛋白的分解代谢和抑制血红素的聚合, 包括抑制食物泡的蛋白水解活性[46]. 此外, 也有研究发现青蒿琥酯可抑制属于膜谷胱甘肽S转移酶的恶性疟原虫抗原EXP1, 该蛋白能有效降解具毒性的高铁血红素[47]. 青蒿素可能有多个分子靶点的发现与其具有快速杀虫的作用是吻合的. 由于寄生虫难以同时发生多个基因的突变, 因此即使广泛地使用青蒿素类药物后也没能产生RⅡ和RⅢ型的抗性. 青蒿素半衰期短的特点可避免药物长时间与寄生虫接触, 从而有利于减小抗药性产生的机率.

4 青蒿素抗性的机制与相关基因

如上所述, 迄今为止还未发现RⅡ和RⅢ型的青蒿素抗性, 关于青蒿素抗性的讨论就仅局限于青蒿素类药物联合疗法后的延迟原虫清除(PC1/2>5 h)、环状体生存试验RSA或复发率的增加. 在这里, “抗性”表示的是这些测试指标中的任何一个. 最近, 位于疟原虫13号染色体编码具有6个螺旋桨结构域的kelch蛋白(“K13”) (PF3D7_1343700)中的M476I的突变被发现与PC1/2>5 h的表型相连锁. 进一步的研究证实, 该蛋白的多个突变与从柬埔寨采集的青蒿素抗性虫株的延迟半数原虫清除时间的增加, 以及0~3 h环状体生存试验存活率的升高具有相关性[48]. 由此kelch蛋白也成为青蒿素抗性的潜在分子标记物. 在东南亚寄生虫群体中存在的K13等位基因突变与体外RSA试验的存活率升高及体内寄生虫清除时间延长的强相关性表明, K13-螺旋桨区的突变可能是青蒿素抗性的决定因素. 有趣的是, 位于染色体13的基因座也与之前收集的患者样本中延迟原虫清除时间的表型相关[49,50]. 自K13的突变型发现以来, K13-螺旋桨区的突变与原虫清除时间延迟的相关性从多个国家搜集的样本中得到了确认, 包括越南、泰国、缅甸和中国[51~55]. 将3种不同的K13-螺旋桨区突变型(C580Y, R539T和I543T)编辑成野生型的序列之后, 其原来的RSA0-3h抗性表型完全丧失, 进一步证实了K13与青蒿素抗性的关系[56]. 因此, K13的突变可增加RⅠ型抗性, 但不能导致RⅡ和RⅢ型抗性.

疟原虫对青蒿素及其衍生物的应答还涉及其他一些基因. 体外进行双氢青蒿素筛选恶性疟原虫可导致*pfmdr1*基因拷贝数和抗氧化水平的增加[57]. *pfmdr1*基因拷贝数减少后会造成寄生虫部分逆转对蒿醚林酸(artelinic acid, AL)的抗性和增加对甲氟喹的易感性[58]. 值得提醒的是, 这两项研究测量的耐药性表型是半致死量IC_{50}或百分九十致死量IC_{90}, 而不是RSA或延迟原虫清除时间, 这意味着具有不同的抗性机制与潜在的RⅡ或RⅢ型抗性. 然而, *pfmdr1*

基因座位的扩增一般不稳定, 在没有药物压力的情况下*pfmdr1*的拷贝数将会发生变化.

全基因组关联研究(genome-wide association studies, GWAS)也鉴定了大量与寄生虫对青蒿素及其衍生物在体外或离体反应相关的基因或遗传位点[59~62]. 尤其令人感兴趣的是, 自噬相关蛋白18 (ATG18)编码基因的突变被发现与双氢青蒿素的敏感性降低有关[62]. 此外, 转录组分析显示青蒿素抗性和未折叠蛋白反应途径表达的增加及环状体发育时间的延长有相关性[63]. 还有, PfATP6, PfPI3K和PfEXP1的突变也可能在寄生虫对青蒿素的应答或抗性中起一定的作用[27,38,47].

5 ACTs与其伴侣药物

许多药物已被用作ACTs中的伴侣药物. 用于治疗恶性疟疾和非恶性疟疾的常见联合疗法包括青蒿琥酯(artesunate, ATS)/乙胺嘧啶-磺胺多辛、青蒿琥酯/阿莫地喹、蒿甲醚(artemether, ATM)/本芴醇、青蒿琥酯/甲氟喹、青蒿琥酯/氯丙胍(proguanil hydrochloride, CPG)或氨苯砜(dapsone, DAP)、青蒿琥酯/阿托伐醌或盐酸氯胍、青蒿琥酯/磷酸咯萘啶、双氢青蒿素/哌喹等[64~66]. 因为青蒿素及其衍生物在体内具有很短的半衰期, 所以选用伴侣药物的标准之一是选择半衰期相对长的药物. 理想的配伍药物将具有相容的药物代谢动力学和药物效应动力学, 具有相异的分子作用模式(modes of action, MOA)和不同的抗性机制, 对现有耐药性寄生虫有效且不增加额外的毒性. 然而, 大多数的ACTs是在没有完全阐明药物与药物间相互作用模式或分子作用模式的情况下开发的, 因此可能不是最理想的组合. 例如, 伴侣药物如甲氟喹、本芴醇等似乎与青蒿素及其衍生物有相似的作用途径, 因此对其中一种药物的敏感性产生的突变型也可以改变对另一种药物的有效性, 导致对两种组合药物的耐受性同时增加[36,43].

为了寻找有效和安全的伴侣药物, Mott等人[67]进行了大规模的药物组合筛查. 不同于每次的测试单一药物, 他们筛选两种药物在不同稀释浓度下的组合. 该研究鉴定了许多与双氢青蒿素联用具有加强作用的药物, 并且建立了药物互作网络, 为评价伴侣药物的药效提供了重要的信息. 有关药物组合的另一个有趣的想法是找到针对寄生虫的相同分子具有相反效果的药物. 例如, *pfcrt*编码序列的突变可以导致疟原虫对氯喹的抗性; 然而, 该基因的突变也可能使寄生虫对其他化合物更为敏感. 这种构想的组合可能提供一种富有前景能同时杀死氯喹敏感性和氯喹抗性疟原虫的方案. 同时, 由于突变的产生受PfCRT的结构和功能限制, 这种药物组合也可能防止新突变型抗性的出现[36]. 针对野生型及突变型的药物组合可以杀灭携带野生型或突变型等位基因的寄生虫. 事实上, Yuan等人[36]在大规模筛选已批准用于人类和动物的药物小分子化合物库时, 也发现了一些对氯喹抗性寄生虫(携带突变体*pfcrt*)更为有效的化合物. 根据这一原则, 使用作用彼此加强的药物组合不一定是好主意. 作用相互加强的药物通常具有类似的分子作用模式和抗性机制; 如果发生的突变导致寄生虫对其中一种药物产生抗性, 第二种药物的有效性也可能丧失. 因而有人提出采用彼此拮抗的药物组合来延长复方药物的使用寿命[68]. 在一些药物组合中, 两种药物联用的效果甚至可能比单方使用的效果更差——这称为超拮抗相互作用或抑制[68]. 拮抗性组合可能需要使用比药物单方更高的剂量, 然而, 这种组合的有益之处在于其具有减少甚至逆转抗性的可能. 当一种药物抗性产生时, 突变型寄生虫可能对其伴侣药物变得更为敏感. 此外, 目前有许多在研发中的新型化合物, 它们将来或许可用作新的抗疟药或作为青蒿素的伴侣药物[69].

青蒿素类复方其伴侣药物的抗性也已有报道. PfMDR1可调节寄生虫对甲氟喹、本芴醇、卤泛群(halofantrine, HAL)等药物的应答[43,44,70,71]. 有报道称, 双氢青蒿素/哌喹复方功效地降低可能是疟原虫对哌喹的易感性下降引起的[72]. 最近, 在柬埔寨的一项调查发现, 编码血红蛋白消化蛋白酶plasmepsin 2-3的拷贝数的扩增与哌喹抗性相关[73]. 位于染色体5靠近*pfmdr1*的一段序列其拷贝数的变异和*pfcrt*的突变也可能影响寄生虫对哌喹的反应[74,75].

6 全民用药与根除疟疾

ACTs用于疟疾治疗的成功也导致了人们对根除疟疾的乐观想法[76~78], 况且有几个研究已取得了良好的效果[79~84]. 在一项研究中, 青蒿素/哌喹加低剂量伯氨喹的药物组合的全民用药方案被用于消灭人体内所有发育阶段的寄生虫[80]. 方案实施3年后, 疟疾感染率从52.3%急剧下降到2.6%, 儿童患恶性疟疾的比率也从37.0%减少到1.4%, 17个村庄中的8个村

落其疟疾感染率为0.

然而, 在推广全民用药之前, 需要考虑许多伦理和各种技术性问题[85,86]. 研究者们要仔细评估全民用药对疟疾传播的长期影响, 特别是对低、中度传播环境的影响, 以及产生耐药性的潜在后果等[78]. 目前, 伯氨喹是唯一可用于杀灭恶性疟原虫配子体并且可预防间日疟原虫和卵形疟原虫复发的抗疟药[87]. 伯氨喹可以与青蒿素联合疗法一起使用来杀配子体以阻断传播; 然而, 低剂量伯氨喹的添加是否可以提高配子体的清除率还需要进一步研究[88]. 此外, 伯氨喹对葡萄糖-6-磷酸脱氢酶(glucose 6-phosphate dehydrogenase, G6PD)缺陷的人群产生的溶血毒性也是大规模全民用药时需关注的问题[87]. 开发新的不引起与伯氨喹相似的溶血毒性、且能阻断疟疾传播的药物可以大大改善用于根除疟疾的全民用药效果[89].

7 结语

青蒿素及其衍生物挽救了无数疟疾患者的生命; 然而, 已报道的延迟原虫清除的现象也敲响了产生青蒿素类药物抗性的警钟. 当前, 最大的担忧是出现RⅡ和RⅢ级别的抗青蒿素的疟原虫. 青蒿素是一种能快速降低原虫血症、缓解症状和预防疟疾相关并发症的良药, 但由于其在体内的半衰期短而不用作预防药物. 青蒿素及其衍生物也已被用于治疗其他寄生虫的感染[90]或其他疾病[91,92]. 尽管目前在青蒿素的药物作用模式、药物靶点和耐药性分子机制等方面的研究已取得了一定的进展, 但是, 对这些问题的认识还远远不够.

致谢 美国国立卫生研究院过敏与传染病研究所的Thomas E. Wellems和Carole A. Long博士给我们提出了建设性的意见和建议, 美国国立卫生研究院图书馆的Cindy Clark对英文文稿进行了校对, 在此一并向他们表示感谢! 最后, 我们要强调的是, 本文阐述的主要进展并未试图涵盖所有与青蒿素相关的研究; 由于篇幅有限, 无法引用许多其他有趣且重要的文献, 为此特表歉意.

参考文献

1 World Health Organization (WHO). World malaria report 2015. http://appswhoint/iris/bitstream/10665/200018/1/9789241565158_engpdf?ua=1, 2015
2 Cowman A F, Healer J, Marapana D, et al. Malaria: Biology and disease. Cell, 2016, 167: 610–624
3 Wassmer S C, Taylor T E, Rathod P K, et al. Investigating the pathogenesis of severe malaria: A multidisciplinary and cross-geographical approach. Am J Trop Med Hyg, 2015, 93: 42–56
4 Cui L, Mharakurwa S, Ndiaye D, et al. Antimalarial drug resistance: Literature review and activities and findings of the ICEMR network. Am J Trop Med Hyg, 2015, 93: 57–68
5 Gaillard T, Madamet M, Pradines B. Tetracyclines in malaria. Malar J, 2015, 14: 445
6 Pantaleo A, Pau M C, Chien H D, et al. Artemisinin resistance, some facts and opinions. J Infect Dev Ctries, 2015, 9: 597–599
7 World Health Organization (WHO). Guielines for the Treatment of Malaria. Geneva: WHO, 2005
8 Nosten F, White N J. Artemisinin-based combination treatment of falciparum malaria. Am J Trop Med Hyg, 2007, 77: 181–192
9 Noedl H, Se Y, Schaecher K, et al. Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med, 2008, 359: 2619–2620
10 Dondorp A M, Nosten F, Yi P, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med, 2009, 361: 455–467
11 Leang R, Taylor W R, Bouth D M, et al. Evidence of *Plasmodium falciparum* malaria multidrug resistance to artemisinin and piperaquine in Western cambodia: Dihydroartemisinin-piperaquine open-label multicenter clinical assessment. Antimicrob Agents Chemother, 2015, 59: 4719–4726
12 World Health Organization (WHO). Chemotherapy of malaria: Report of a WHO scientific group. Technical Report, World Health Organization, 1967
13 Fidock D A, Nomura T, Talley A K, et al. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Mol Cell, 2000, 6: 861–871
14 Peterson D S, Milhous W K, Wellems T E. Molecular basis of differential resistance to cycloguanil and pyrimethamine in *Plasmodium falciparum* malaria. Proc Natl Acad Sci USA, 1990, 87: 3018–3022
15 Djimde A, Doumbo O K, Cortese J F, et al. A molecular marker for chloroquine-resistant falciparum malaria. N Engl J Med, 2001, 344: 257–263

16 WWARN Parasite Clearance Study Group, Abdulla S, Ashley E A, et al. Baseline data of parasite clearance in patients with falciparum malaria treated with an artemisinin derivative: An individual patient data meta-analysis. Malar J, 2015, 14: 359
17 Fairhurst R M. Understanding artemisinin-resistant malaria: What a difference a year makes. Curr Opin Infect Dis, 2015, 28: 417–425
18 Amaratunga C, Neal A T, Fairhurst R M. Flow cytometry-based analysis of artemisinin-resistant *Plasmodium falciparum* in the ring-stage survival assay. Antimicrob Agents Chemother, 2014, 58: 4938–4940
19 Witkowski B, Amaratunga C, Khim N, et al. Novel phenotypic assays for the detection of artemisinin-resistant *Plasmodium falciparum* malaria in Cambodia: *In-vitro* and *ex-vivo* drug-response studies. Lancet Infect Dis, 2013, 13: 1043–1049
20 Amaratunga C, Sreng S, Suon S, et al. Artemisinin-resistant *Plasmodium falciparum* in Pursat province, western Cambodia: A parasite clearance rate study. Lancet Infect Dis, 2012, 12: 851–858
21 Li G Q, Arnold K, Guo X B, et al. Randomised comparative study of mefloquine, qinghaosu, and pyrimethamine-sulfadoxine in patients with falciparum malaria. Lancet, 1984, 2: 1360–1361
22 Spring M D, Lin J T, Manning J E, et al. Dihydroartemisinin-piperaquine failure associated with a triple mutant including kelch13 C580Y in Cambodia: An observational cohort study. Lancet Infect Dis, 2015, 15: 683–691
23 Leang R, Barrette A, Bouth D M, et al. Efficacy of dihydroartemisinin-piperaquine for treatment of uncomplicated *Plasmodium falciparum* and *Plasmodium vivax* in Cambodia, 2008 to 2010. Antimicrob Agents Chemother, 2013, 57: 818–826
24 Duru V, Witkowski B, Menard D. *Plasmodium falciparum* resistance to artemisinin derivatives and piperaquine: A major challenge for malaria elimination in Cambodia. Am J Trop Med Hyg, 2016, 95: 1228–1238
25 Lu F, Culleton R, Zhang M, et al. Emergence of indigenous artemisinin-resistant *Plasmodium falciparum* in Africa. N Engl J Med, 2017, 376: 991–993
26 Meunier B, Robert A. Heme as trigger and target for trioxane-containing antimalarial drugs. Acc Chem Res, 2010, 43: 1444–1451
27 Eckstein-Ludwig U, Webb R J, Van Goethem I D, et al. Artemisinins target the SERCA of *Plasmodium falciparum*. Nature, 2003, 424: 957–961
28 Stocks P A, Bray P G, Barton V E, et al. Evidence for a common non-heme chelatable-iron-dependent activation mechanism for semisynthetic and synthetic endoperoxide antimalarial drugs. Angew Chem Int Ed Engl, 2007, 46: 6278–6283
29 Meshnick S R. The mode of action of antimalarial endoperoxides. Trans R Soc Trop Med Hyg, 1994, 88(Suppl 1): S31–S32
30 Klonis N, Crespo-Ortiz M P, Bottova I, et al. Artemisinin activity against *Plasmodium falciparum* requires hemoglobin uptake and digestion. Proc Natl Acad Sci USA, 2011, 108: 11405–11410
31 Wang J, Zhang C J, Chia W N, et al. Haem-activated promiscuous targeting of artemisinin in *Plasmodium falciparum*. Nat Commun, 2015, 6: 10111
32 Arnou B, Montigny C, Morth J P, et al. The *Plasmodium falciparum* Ca^{2+}-ATPase PfATP6: Insensitive to artemisinin, but a potential drug target. Biochem Soc Trans, 2011, 39: 823–831
33 Adhin M R, Labadie-Bracho M, Vreden S G. Status of potential PfATP6 molecular markers for artemisinin resistance in Suriname. Malar J, 2012, 11: 322
34 Brasil L W, Areas A L, Melo G C, et al. Pfatp6 molecular profile of *Plasmodium falciparum* isolates in the western Brazilian Amazon. Malar J, 2012, 11: 111
35 Pulcini S, Staines H M, Pittman J K, et al. Expression in yeast links field polymorphisms in PfATP6 to *in vitro* artemisinin resistance and identifies new inhibitor classes. J Infect Dis, 2013, 208: 468–478
36 Yuan J, Cheng K C, Johnson R L, et al. Chemical genomic profiling for antimalarial therapies, response signatures, and molecular targets. Science, 2011, 333: 724–729
37 Eastman R T, Khine P, Huang R, et al. PfCRT and PfMDR1 modulate interactions of artemisinin derivatives and ion channel blockers. Sci Rep, 2016, 6: 25379
38 Mbengue A, Bhattacharjee S, Pandharkar T, et al. A molecular mechanism of artemisinin resistance in *Plasmodium falciparum* malaria. Nature, 2015, 520: 683–687
39 Bhisutthibhan J, Pan X Q, Hossler P A, et al. The *Plasmodium falciparum* translationally controlled tumor protein homolog and its reaction with the antimalarial drug artemisinin. J Biol Chem, 1998, 273: 16192–16198
40 Ismail H M, Barton V, Phanchana M, et al. Artemisinin activity-based probes identify multiple molecular targets within the asexual stage of the malaria parasites *Plasmodium falciparum* 3D7. Proc Natl Acad Sci USA, 2016, 113: 2080–2085
41 Cooper R A, Ferdig M T, Su X Z, et al. Alternative mutations at position 76 of the vacuolar transmembrane protein PfCRT are associated with chloroquine resistance and unique stereospecific quinine and quinidine responses in *Plasmodium falciparum*. Mol Pharmacol, 2002, 61: 35–42
42 Sidhu A B, Verdier-Pinard D, Fidock D A. Chloroquine resistance in *Plasmodium falciparum* malaria parasites conferred by *pfcrt* mutations. Science, 2002, 298: 210–213

43 Sidhu A B, Uhlemann A C, Valderramos S G, et al. Decreasing pfmdr1 copy number in *Plasmodium falciparum* malaria heightens susceptibility to mefloquine, lumefantrine, halofantrine, quinine, and artemisinin. J Infect Dis, 2006, 194: 528–535

44 Reed M B, Saliba K J, Caruana S R, et al. Pgh1 modulates sensitivity and resistance to multiple antimalarials in *Plasmodium falciparum*. Nature, 2000, 403: 906–909

45 Zhou Y, Li W, Xiao Y. Profiling of multiple targets of artemisinin activated by hemin in cancer cell proteome. ACS Chem Biol, 2016, 11: 882–888

46 Pandey A V, Tekwani B L, Singh R L, et al. Artemisinin, an endoperoxide antimalarial, disrupts the hemoglobin catabolism and heme detoxification systems in malarial parasite. J Biol Chem, 1999, 274: 19383–19388

47 Lisewski A M, Quiros J P, Ng C L, et al. Supergenomic network compression and the discovery of EXP1 as a glutathione transferase inhibited by artesunate. Cell, 2014, 158: 916–928

48 Ariey F, Witkowski B, Amaratunga C, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature, 2014, 505: 50–55

49 Takala-Harrison S, Clark T G, Jacob C G, et al. Genetic loci associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment in Southeast Asia. Proc Natl Acad Sci USA, 2013, 110: 240–245

50 Cheeseman I H, Miller B A, Nair S, et al. A major genome region underlying artemisinin resistance in malaria. Science, 2012, 336: 79–82

51 Ashley E A, Dhorda M, Fairhurst R M, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med, 2014, 371: 411–423

52 Huang F, Takala-Harrison S, Jacob C G, et al. A single mutation in K13 predominates in southern china and is associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment. J Infect Dis, 2015, 212: 1629–1635

53 Nyunt M H, Hlaing T, Oo H W, et al. Molecular assessment of artemisinin resistance markers, polymorphisms in the k13 propeller, and a multidrug-resistance gene in the eastern and western border areas of Myanmar. Clin Infect Dis, 2015, 60: 1208–1215

54 Takala-Harrison S, Jacob C G, Arze C, et al. Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia. J Infect Dis, 2015, 211: 670–679

55 Tun K M, Imwong M, Lwin K M, et al. Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: A cross-sectional survey of the K13 molecular marker. Lancet Infect Dis, 2015, 15: 415–421

56 Straimer J, Gnadig N F, Witkowski B, et al. Drug resistance. K13-propeller mutations confer artemisinin resistance in *Plasmodium falciparum* clinical isolates. Science, 2015, 347: 428–431

57 Cui L, Wang Z, Miao J, et al. Mechanisms of *in vitro* resistance to dihydroartemisinin in *Plasmodium falciparum*. Mol Microbiol, 2012, 86: 111–128

58 Chen N, Chavchich M, Peters J M, et al. Deamplification of *pfmdr1*-containing amplicon on chromosome 5 in *Plasmodium falciparum* is associated with reduced resistance to artelinic acid *in vitro*. Antimicrob Agents Chemother, 2010, 54: 3395–3401

59 Mu J, Myers R A, Jiang H, et al. *Plasmodium falciparum* genome-wide scans for positive selection, recombination hot spots and resistance to antimalarial drugs. Nat Genet, 2010, 42: 268–271

60 Miotto O, Almagro-Garcia J, Manske M, et al. Multiple populations of artemisinin-resistant *Plasmodium falciparum* in Cambodia. Nat Genet, 2013, 45: 648–655

61 Miotto O, Amato R, Ashley E A, et al. Genetic architecture of artemisinin-resistant *Plasmodium falciparum*. Nat Genet, 2015, 47: 226–234

62 Wang Z, Cabrera M, Yang J, et al. Genome-wide association analysis identifies genetic loci associated with resistance to multiple antimalarials in *Plasmodium falciparum* from China-Myanmar border. Sci Rep, 2016, 6: 33891

63 Mok S, Ashley E A, Ferreira P E, et al. Drug resistance. Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. Science, 2015, 347: 431–435

64 Nosten F, White N J. Artemisinin-based combination treatment of falciparum malaria. In: Breman J G A M, White N J, eds. Defining and Defeating the Intolerable Burden of Malaria III: Progress and Perspectives. Northbrook: American Society of Tropical Medicine and Hygiene, 2007

65 Visser B J, Wieten R W, Kroon D, et al. Efficacy and safety of artemisinin combination therapy (ACT) for non-falciparum malaria: A systematic review. Malar J, 2014, 13: 463

66 Visser B J, van Vugt M, Grobusch M P. Malaria: An update on current chemotherapy. Expert Opin Pharmacother, 2014, 15: 2219–2254

67 Mott B T, Eastman R T, Guha R, et al. High-throughput matrix screening identifies synergistic and antagonistic antimalarial drug combinations. Sci Rep, 2015, 5: 13891

68 Chait R, Craney A, Kishony R. Antibiotic interactions that select against resistance. Nature, 2007, 446: 668–671

69 Wells T N, Hooft van Huijsduijnen R, Van Voorhis W C. Malaria medicines: A glass half full? Nat Rev Drug Discov, 2015, 14: 424–442

70 Lim P, Dek D, Try V, et al. Decreasing pfmdr1 copy number suggests that *Plasmodium falciparum* in Western Cambodia is regaining *in vitro* susceptibility to mefloquine. Antimicrob Agents Chemother, 2015, 59: 2934–2937

71 Veiga M I, Dhingra S K, Henrich P P, et al. Globally prevalent PfMDR1 mutations modulate *Plasmodium falciparum* susceptibility to artemisinin-based combination therapies. Nat Commun, 2016, 7: 11553

72 Amaratunga C, Lim P, Suon S, et al. Dihydroartemisinin-piperaquine resistance in *Plasmodium falciparum* malaria in Cambodia: A multisite prospective cohort study. Lancet Infect Dis, 2016, 16: 357–365

73 Witkowski B, Duru V, Khim N, et al. A surrogate marker of piperaquine-resistant *Plasmodium falciparum* malaria: A phenotype-genotype association study. Lancet Infect Dis, 2017, 17: 174–183

74 Eastman R T, Dharia N V, Winzeler E A, et al. Piperaquine resistance is associated with a copy number variation on chromosome 5 in drug-pressured *Plasmodium falciparum* parasites. Antimicrob Agents Chemother, 2011, 55: 3908–3916

75 Gabryszewski S J, Dhingra S K, Combrinck J M, et al. Evolution of fitness cost-neutral mutant PfCRT conferring *P. falciparum* 4-aminoquinoline drug resistance is accompanied by altered parasite metabolism and digestive vacuole physiology. PLoS Pathog, 2016, 12: e1005976

76 Maude R J, Socheat D, Nguon C, et al. Optimising strategies for *Plasmodium falciparum* malaria elimination in Cambodia: Primaquine, mass drug administration and artemisinin resistance. PLoS One, 2012, 7: e37166

77 Kern S E, Tiono A B, Makanga M, et al. Community screening and treatment of asymptomatic carriers of *Plasmodium falciparum* with artemether-lumefantrine to reduce malaria disease burden: A modelling and simulation analysis. Malar J, 2011, 10: 210

78 Poirot E, Skarbinski J, Sinclair D, et al. Mass drug administration for malaria. Cochrane Database Syst Rev, 2013, 9: CD008846

79 Hsiang M S, Hwang J, Tao A R, et al. Mass drug administration for the control and elimination of *Plasmodium vivax* malaria: An ecological study from Jiangsu Province, China. Malar J, 2013, 12: 383

80 Song J, Socheat D, Tan B, et al. Rapid and effective malaria control in Cambodia through mass administration of artemisinin-piperaquine. Malar J, 2010, 9: 57

81 Lwin K M, Imwong M, Suangkanarat P, et al. Elimination of *Plasmodium falciparum* in an area of multi-drug resistance. Malar J, 2015, 14: 319

82 Newby G, Hwang J, Koita K, et al. Review of mass drug administration for malaria and its operational challenges. Am J Trop Med Hyg, 2015, 93: 125–134

83 Silumbe K, Yukich J O, Hamainza B, et al. Costs and cost-effectiveness of a large-scale mass testing and treatment intervention for malaria in Southern Province, Zambia. Malar J, 2015, 14: 211

84 Eisele T P, Bennett A, Silumbe K, et al. Short-term impact of mass drug administration with dihydroartemisinin plus piperaquine on malaria in Southern Province Zambia: A cluster-randomized controlled trial. J Infect Dis, 2016, 214: 1831–1839

85 Jamrozik E, de la Fuente-Nunez V, Reis A, et al. Ethical aspects of malaria control and research. Malar J, 2015, 14: 518

86 Cheah P Y, White N J. Antimalarial mass drug administration: Ethical considerations. Int Health, 2016, 8: 235–238

87 Ashley E A, Recht J, White N J. Primaquine: The risks and the benefits. Malar J, 2014, 13: 418

88 El-Sayed B, El-Zaki S E, Babiker H, et al. A randomized open-label trial of artesunate-sulfadoxine-pyrimethamine with or without primaquine for elimination of sub-microscopic *P. falciparum* parasitaemia and gametocyte carriage in eastern Sudan. PLoS One, 2007, 2: e1311

89 Eastman R T, Pattaradilokrat S, Raj D K, et al. A class of tricyclic compounds blocking malaria parasite oocyst development and transmission. Antimicrob Agents Chemother, 2013, 57: 425–435

90 Ni Loo C S, Kei Lam N S, Yu D, et al. Artemisinin and its derivatives in treating protozoan infections beyond malaria. Pharmacol Res, 2017, 117: 192–217

91 Kim S H, Kang S H, Kang B S. Therapeutic effects of dihydroartemisinin and transferrin against glioblastoma. Nutr Res Pract, 2016, 10: 393–397

92 Chen G, Gong R, Shi X, et al. Halofuginone and artemisinin synergistically arrest cancer cells at the G1/G0 phase by upregulating $p21^{Cip1}$ and $p27^{Kip1}$. Oncotarget, 2016, 7: 50302–50314

Summary for "青蒿素与疟疾: 药物激活、作用机理及抗药性的研究进展"

Artemisinin and malaria: Current understandings of drug activation, action, and resistance[a)]

SU XinZhuan[1,2*] & LI Jian[2]

[1] *Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda 20892, USA;*
[2] *State Key Laboratory of Cellular Stress Biology, Innovation Center for Cell Signaling Network, School of Life Sciences, Xiamen University, Xiamen 361005, China*
* Corresponding author, E-mail: xsu@niaid.nih.gov

Malaria is a disease that can be cured with antimalarial drugs if treated early and appropriately. However, parasites resistant to a new drug generally emerge within a few years after large-scale applications. Artemisinin (ART or Qinghaosu) combination therapies (ACTs) have become the major treatments for malaria after the emergence of parasites resistant to almost all classes of antimalarial drugs. Parasites with delayed parasite clearance (DPC) after ART or ACT therapies have also been reported in Southeast Asia, raising concerns of total failure of ART and its derivatives.

Many classes of antimalarial drugs have been introduced to successfully treat malaria infections, including chloroquine, piperaquine, primaquine, mefloquine (MQ), pyrimethamine, sulfadoxine, ART and derivatives, etc. Regrettably, many of these drugs have been abandoned by many countries in malaria endemic regions due to the emergence of drug resistant parasites. According to World Health Organization, parasite responses to a drug can be classified into four categories (S, RI, RII, and RIII): Sensitivity (S) to a drug is defined as clearance of asexual parasitemia within seven days of the first day of treatment without recrudescence; Resistance RI is defined as clearance of asexual parasitemia as in sensitive parasites, followed by recrudescence; RII resistance is indicated by marked reduction of asexual parasitemia, but no clearance; and RIII resistance shows no marked reduction of asexual parasitemia. Currently, RII and RIII resistance to chloroquine, pyrimethamine, and other drugs have been widely reported; but residence to ART remains largely at R1 level. ART resistance was initially defined as parasites with half parasite clearance time (PC1/2)>5 h under a standard ART treatment regimen (three-day artesunate treatment at 2–4 mg kg^{-1} d^{-1}). A second measurement is *in vitro* ring survival assay (RSA) that was developed based on the observation that the ring stages of some parasite strains could survive a short period of ART treatment. Another indicator of increasing ART tolerance is the elevated rate of recrudescence after ART or ACT treatments.

The generation of highly reactive radicals via endoperoxide cleavage is critical for ART activation. Both free ferrous iron and heme have been proposed to be the predominant iron sources for ART activation. The heme required for ART activation can be derived from the parasite's heme biosynthesis pathway at the early ring stage and/or from hemoglobin digestion at later stages. A large number of parasite molecules have been found to bind or interact with ART, most notably the *Plasmodium falciparum* ATPase 6 (PfATP6 or SERCA), phosphatidylinositol-3-kinase (PfPI3K), chloroquine resistance transporter (PfCRT), and multiple drug resistance 1 (PfMDR1). Recently, a gene encoding a parasite Kelch protein ("K13") with a six-blade propeller domain was identified as a potential molecular marker of ART resistance *in vivo* (DPC>5 h) and *in vitro* (RSA).

Various antimalarial drugs such as meflouine and piperaquine have been used as partner drugs in ACTs. However, parasites resistant to these partner drugs have also been reported, which may explain the reported slow parasite clearance after ACT treatment. The success of ACTs in treating malaria infections has generated optimism and proposals for malaria eradication by mass drug administration (MDA), and successes have been achieved from several studies. However, the impact of MDA on malaria transmission in the long term, especially in low- and moderate-transmission settings, and the potential consequences of developing drug resistance, requires careful evaluation.

There are a large number of studies and publications on these related subjects, and it is impossible to include or cite all the publications in this review. Additionally, some of the issues discussed here are still being debated, requiring further investigation.

Qinghaosu, *Plasmodium*, drug combination, mass drug administration (MDA), artemisinin combination therapy (ACT)

doi: 10.1360/N972017-00147

a) More information see "Supplementary materials online"