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论著

酮替芬和赛庚啶增强体外培养的恶性疟原虫氯喹抗性株对氯喹反应性的研究

权红,汤林华*

中国疾病预防控制中心寄生虫病预防控制所，世界卫生组织疟疾、血吸虫病和丝虫病合作中心，上海 200025

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摘要

目的 研究酮替芬和赛庚啶增强体外培养的恶性疟原虫氯喹抗性株对氯喹反应性，及其增效机制。 方法 恶性疟原虫氯喹抗性株(Fcc SM1/yN株)取自云南省思茅地区恶性疟患者静脉血，经同步化处理，用新鲜血将红细胞感染率调至0.5%～1.0%，红细胞压积与培养基体积比(红细胞比容)为1 ： 9，混匀。制备氯喹药板及氯喹/酮替芬（或氯喹/赛庚啶）组合药板。氯喹药板自上而下每行氯喹终浓度依次为0.312 5～2 560 nmol/L，呈2倍递增。氯喹/酮替芬（或氯喹/赛庚啶）组合药板，是在氯喹药板基础上加入酮替芬（或赛庚啶），每行10孔自左至右终浓度依次为9.77～5 000 nmol/L，呈2倍递增，每块药板均设A行空白对照。每孔加混匀血样 50 μl，37 ℃ 培养 34 h，镜检计数每200个疟原虫中含3个核以上裂殖体数，计算氯喹单药及各配伍组对恶性疟原虫的半数抑制浓度（IC_{50}），以及酮替芬（或赛庚啶）提高氯喹活性的指数（AEI）。选择AEI值较高的配伍组，进行增效时序性研究。当氯喹对虫体作用 0～10 h 分别加入酮替芬（或赛庚啶），34 h后检测和计算各时间段IC_{50}及AEI。选择氯喹/酮替芬（或氯喹/赛庚啶）最佳配伍剂量，培养恶性疟原虫 20 h，提取总RNA，用实时荧光定量PCR(real-time PCR)分析药物作用前后恶性疟原虫氯喹抗性转移基因（*pfcrt*）和多药抗性基因（*pfmdr*1）表达水平。 结果 0.312 5～2 560 nmol/L氯喹与625 nmol/L 酮替芬（或赛庚啶）配伍，增效作用显著，氯喹/酮替芬的IC50为74.53 nmol/L，AEI为0.42；氯喹/赛庚啶的IC50为89.70 nmol/L、AEI为0.30。5 nmol/L氯喹作用 6～7 h 加入625 nmol/L酮替芬（或赛庚啶），增效作用显著，氯喹/酮替芬的IC50为 67.70 nmol/L、AEI为0.47；氯喹/赛庚啶的IC_{50}为81.53 nmol/L、AEI为0.37。5 nmol/L氯喹与625 nmol/L酮替芬（或赛庚啶）配伍，作用20 h，氯喹/酮替芬可使*pfcrt*基因表达水平升高91%，而氯喹/赛庚啶可使*pfmdr*1 基因表达水平下降14%。 结论 体外氯喹与适量的酮替芬（或赛庚啶）配伍，能增强恶性疟原虫氯喹抗性株对氯喹的反应性。氯喹对虫体作用 6～7 h 加入酮替芬（或赛庚啶）增效作用显著。该增效作用与*pfcrt*基因和*pfmdr*1 基因的表达水平有关。

关键词 恶性疟原虫 抗性 氯喹 酮替芬 赛庚啶 *pfcrt*基因 *pfmdr*1基因 基因表达

分类号

In vitro Potentiation of Chloroquine Activity in *Plasmodium falciparum* by Ketotifen and Cyproheptadine

QUAN Hong,TANG Lin-hua*

National Institute of Parasitic Diseases，Chinese Center for Disease Control and Prevention，WHO Collaborating Centre for Malaria，Schistosomiasis and Filariasis，Shanghai 200025，China

Abstract

Objective To study the potentiation of chloroquine activity and mechanism by ketotifen and cyproheptadine in *in vitro* cultured *Plasmodium falciparum* Fcc SM1/yN strain. Methods *In vitro* cultured Fcc SM1/yN strain was added to pre-prepared drug plates at 50 μl/well after synchronization to make final concentration of 0.312 5-2 560 nmol/L for chloroqine and of 9.80-5 000 nmol/L for ketotifen or cyproheptadine. After 34 hours' culture in 37 ℃，the number of schizonts with 3 or more nuclei was calculated among 200 parasites under microscope. Calculated half inhibitive concentration (IC_{50}) of chloroquine and every drug combination to parasite as well as chloroquine activity enhancement index (AEI) of ketotifen (or cyproheptadin). Time dependency of potentiation was studied. All data were analyzed statistically with SPSS 13.0. After 20 hours' action of one optimal combination dose of chloroquine/ketotifen or chloroquine/cyproheptadine，RNA of the Fcc SM1/yN strain was extracted and real-time PCR was used to determine the expression level of *pfcrt* and *pfmdr*1 genes. Results The best potentiation effect was observed with ketotifen or cyproheptadine of 625 nmol/L，with IC_{50} of 74.53 nmol/L for chloroquine/ketotifen and 89.7 nmol/L for chloroquine/cyproheptadine respectively，and activity enhancement index (AEI) of 0.42 for chloroquine/ketotifen and 0.30 for chloroquine/cyproheptadine respectively. Combination of 625 nmol/L ketotifen or cyproheptadine with 5 nmol/L chloroquine

showed the highest potentiation potency. 6-7 hours during which ketotifen or cyproheptadine was added after chloroquine showed the highest effect，with IC_{50} of 67.70 nmol/L for chloroquine/ketotifen and 81.53 nmol/L for chloroquine/cyproheptadine respectively，and the AEI was 0.47 for chloroquine/ketotifen and 0.37 for chloroquine/cyproheptadine respectively. After action of chloroquine/ketotifen or chloroquine/cyproheptadine at one optimal combination dose，expression level of *pfcrt* gene increased by 91% and that of *pfmdr*1 gene decreased by 14% respectively. Conclusion Appropriate combination of chloroquine/ketotiphen or chloroquine/cyproheptadine potentiates chloroquine against *in vitro* cultured *P. falciparum*. 6-7 hour period is an optimal time when ketotifen or cyproheptadine was added after chloroquine. Potentiating activity of ketotifen and cyproheptadine may be related to the expression level of *pfcrt* and *pfmdr*1 genes.
Key words *Plasmodium alciparum* Resistance Chloroquine Ketotifen Cyproheptadine *pfcrt* gene *pfmdr*1 gene Gene expression

DOI:

通讯作者 汤林华 ipdtlh@public3.sta.net.cn

作者个人主页 权红;汤林华*