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Original Article

Comparison of the antimicrobial activity of the curcumin derivatives between Fm0817 and Fm04

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Abstract: The objectives were to analyze the anti-bacterial activity of the curcumin derivative FM0817 and FM04 and the mechanism of action. We first tested 40 *Neisseria gonorrhoeaeN. Gonorrhoeae* strains of their susceptibilities to antibiotics by the Kirby-Bauer (KB) methods. DNA was extracted by the boiling method, and 22 strains of *N. gonorrhoeae* were randomly selected and used as templates for PCR amplification of the antibiotic-resistance genes: *TEM*, *tetM*, *erm,* and *mefA*. The filter disc diffusion method was used to determine whether FM0817 and FM04 had anti-bacterial activity, and compare the Antimicrobial activity of the curcumin derivatives between FM0817 and FM04. The susceptibility tests confirmed multi-drug resistance in *N. gonorrhoeae*. The percentage of strains possessing *TEM* and *tetM* was 77.2% and 77.2%, respectively. The drug-resistance genes, *erm* and *mefA*, were not detected in any of the strains studied. FM0817 exhibited potent anti-bacterial activity and FM04 showed feed action *in vitro*, but the relationship between FM0817 and FM04 activity and the resistance genes of *N. gonorrhoeae* remains unclear, therefore, it requires further investigation.

Keywords: Curcumin, anti-bacterial activity, neisseria gonorrhoeae, resistance genes

Introduction

The drug resistance varies greatly among countries. Therefore having prevalence's data as well as the drug susceptibility pattern within consecutive year is important especially for gonorrhea, the highly drug resistant bacteria [1]. Looking for a new drug neisseria gonorrhoeae is imminent.

N, gonorrhoeae has developed resistance to all antimicrobials previously recommended as first-line treatment of gonorrhea, e.g. penicillins, tetracyclines and fluoroquinolones [2]. However, consistent worldwide increases of the resistance gonorrhea to fluoroquinolone have made cephalosporine the only recommended drug for treatment since 2010 [3]. Due to the reduction in treatment options for *N. gonorrhoeae*, alternative anti-microbial materials are urgently needed to maintain control of gonorrhea infections.

Curcumin is a yellow-orange polyphenol compound produced by the rhizome of Curcuma longa plants, which is widely used as a spice in Asian cooking. This compound has been shown to possess a wide range of pharmacological activities [4]. The water-solubility of ginger flavine is poor, but hydrolysis can be carried out in peroxide solution. These factors have limited its biological application and have caused an industrial production bottleneck. Studies have been performed to modify the ginger flavine structure by retaining the ginger flavine aromatic ring structure and replacing the single-carbonyl piperidine hydrochloride 42, but not the saturated B2 two alkones, resulting in the synthesis of a new ginger flavine derivative, designated FM0817 [5, 6], and retain the saturated B2 two alkones, resulting in the synthesis of another new ginger flavine derivative, designated FM04. In this study, we assessed the potential anti-bacterial activities of the curcumin derivative FM04 and FM0817 on *N. gonorrhoeae*, along with their mechanism of action.

Table 1. Primers used to amplify *Neisseria gonorrhoeae* drug-resistant genes

Drug-resistance gene	Primer sequence	Product size (bp)
TEM gene exon	P1: 5'-ATAAAATTCTTGAAAACGAAA-3' P2: 5'-GACAGTTACCAATGCTTAATCA-3'	1074
TEM gene within exon	P3: 5'-AGGAAGAGTATGATTCAACA-3' P4: 5'-CTCGTCGTTTGGTATGGC-3'	535
tetM	P1: 5'-GTGGACGAACTTTACCGAA-3' P2: 5'-GCTTTGTATCTCCAAGAACAC-3'	501
erm	P1: 5'-GGATACGGTTTAGATATTGGG-3' P2: 5'-TTGAAGGACAATGGAACCTCC-3'	295
mefA	P1: 5'-ACTATCATTAATCACTAGTGC-3' P2: 5'-TTCTTCTGGTACTAAAAGTGG-3'	346

Methods

Specimen origin and gonococcus isolation

Forty strains of *N. gonorrhoeae* were isolated from specimens between January 2009 and August 2010 from Zhongshan People's Hospital received by the hospital. *N. gonorrhoeae* were identified by API NH.

Drug sensitive analyses

The drug resistance of forty gonococcal isolates were tested according to the National Center for Clinical Laboratory (China) using the Kirby-Bauer (KB) method. The results were interpreted according to the standard reference, CLSI 2010 [7]. The reference strain, ATCC 49226, was used as the positive control. Among these strains, twenty-two strains were analyzed of the drug genes and the antimicrobial activity of the curcumin derivative FM04 and FM0817.

Sequence of drug-resistant genes primers

Bacterial DNA was extracted according to the previously published boiling method [8]. Previously reported primers were used in this study [9], as shown in **Table 1**. Primers were synthesized by Boya Biological Corporation.

PCR amplification

PCR reaction was total volume of 20 μl using 2 x Premix Taq Version 2.0 (Loading dye Mix) (TakaRa, China). The amplification parameters consisted of a pre-denaturation step at 94°C for 10 min, followed by 25 cycles of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C, with a final 10 min at 72°C in C1000™ Thermal Cycler (BIO-RAD, America). PCR products were analyzed by electrophoresis on a 1.5% agarose gel against a 1500 bp DNA ladder (TakaRa, China). The *TEM* gene from *Escherichia coli* and the *tetM* gene from *Staphylococcus aureus* were used as positive control genes.

Anti-bacterial activity

The KB method was used to determine the antibacterial activity of FM04 and FM0817. The inoculums were prepared in special saline in 0.5 McFarland standard (ca. 1.5×10^8 colony-forming units, cfu/ml) and were cultured on Mueller-Hinton agar plate. Every strain was inoculated in three Mueller-Hinton agar plates, respectively. Three kinds of discs were used in each Mueller-Hinton agar, the bank, curcumin and curcumin derivatives FM04 or FM0817. The experiment was repeated three times. The blank disks, soaked in sterile water, were applied as the control disk. The dishes were then incubated in a 5-10% thermostatic CO_2 cultivation cabinet, for 18-24 h at 35°C. The diameters of inhibition zones were measured and the average values were recorded.

Statistical analysis

The inhibition zones among different drug-resistance gene mutations were analyzed using the SPSS software.

Results

Drug sensitivity of the N. gonorrhoeae strains

All of the forty *N. gonorrhoeae* strains tested were resistant to penicillin. All strains were susceptible to spectinomycin and cefuroxime axetil. The levofloxacin-, ciprofloxacin-, and tetracycline-resistance rates of the strains were 95.0%, 92.5%, and 62.5%, respectively, as shown in **Table 2**. *N. gonorrhoeae* strains, representing eight antibiotic-resistance phenotypes, were divided into five groups based on drug-resistance model.

Table 2. Kirby-Bauer anti-microbial susceptibility testing of *N. gonorrhoeae* strains isolated from January 2009 to August 2010 in Zhongshan district (*n* = 40)

Antibiotic	% R (Resistance rate)	% I (Mid-sensitive rate)	% S (Sensitive rate)
Cefoxitin	10 (4/40)	2.5 (1/40)	87.5 (35/40)
Cefuroxime axetil	0 (0/40)	7.5 (3/40)	92.5 (37/40)
Ciprofloxacin	92.5 (37/40)	7.5 (3/40)	0 (0/29)
Ceftriaxone	5.0 (2/40)	2.5 (2/40)	92.5 (37/40)
Ofloxacin	95.0 (38/40)	5.0 (2/40)	0 (0/40)
Penicillin	75.0 (30/40)	25.0 (10/29)	0 (0/40)
Spectinomycin	0 (0/40)	2.5 (1/40)	97.5 (39/40)
Tetracycline	62.5 (25/40)	25.0 (10/29)	12.5 (5/40)

Table 3. FM0817 and FM04 *in vitro* experiments with selected strains of *N. gonorrhoeae* (*n* = 22)

Genotype	Number of strains (%)	Average of diameters of bacteriostatic annulus (mm)#			
		FM0817	FM04	The blank	Curcumin
TEM- tetM-	1 (4.7)	36.0	9.0	6	6
TEM+ tetM+	13 (59.1)	36.5	9.0	6	6
TEM- tetM+	4 (18.1)	35.6	10.0	6	6
TEM+ tetM-	4 (18.1)	36.0	9.5	6	6

#The diameter of 6.0 mm is included in the inhibition zone disc ammeter.

Prevalence of drug-resistance genes

The four drug-resistance genes of twenty-two strains were detected in this study. The gene-mutation rates of *TEM* and *tetM* were both 77.2%, and the *erm* and *mefA* genes were not detected (**Table 3**). These data showed the enzymes were responsible to the penicillin resistance. The drug-resistance rate of Tetracycline was high (62.5%), but there was no enzyme detected. There were other drug-resistance mechanisms. Twenty-two *N. gonorrhoeae* strains tested were divided to four mutation types. Thirteen strains were detected both *TEM* and *tetM* genes (59.1%). Four strains were detected *TEM* gene (18.1%). Four strains were detected *tetM* gene (18.1%). There was one strains what were not drug-resistance genes detected (4.7%).

Anti-bacterial activity

All of the twenty-two strains were tested the antimicrobial susceptibility of FM04 and FM0817 (**Table 3**). The bacteriostatic annulus diameters of between 33-37 mm of the curcumin derivative FM0817 were observed, however, the bacteriostatic annulus diameters of the curcumin derivative FM04 were only 8-11 mm observed, lower than that of FM0817. The blank disc and the curcumin disc were not inhibition zone. It indicated that both of the FM04 and the FM0817 had the activity of inhibiting *N. gonorrhoeae*. However, no significant differences were detected about the inhibition zones among different drug-resistance gene mutations. Taken together these data suggested that there was no relation between the inhibition activity and the mutation kinds.

Discussion

In the present study, the susceptibility of the antimicrobial drugs was studied between January 2009 and August 2010. Exceedingly high prevalence of resistance was observed for previous first-line antimicrobial such as ciprofloxacin (92.5%), and tetracycline (62.5%). These data are largely in accordance with previous surveys in the Southeast Asian region. In Sri Lanka, 97% and 8.2% resistance have been reported to penicillin and ciprofloxacin, respectively, and in Bangladesh resistance to ciprofloxacin, penicillin, and tetracycline was found to be 76%, 33% and 57%, respectively [10]. Finally, in Pakistan 92%, 87%, and 78% resistance to ofloxacin, penicillin G, and tatracyline, respectively, has been noted [11]. The detection rates for penicillin- and tetracycline-resistance genes recorded in this study were significantly higher than those reported by Li et al. [12], likely due to the widespread application of antibiotics and the rapid spread of drug-resistance genes. The erythromycin-resistance-related methylation enzyme, encoded by the *erm* gene and the *mefA* gene, was not detected in our study. Analysis of the prevalence of drug resistance among *N. gonorrhoeae* strains throughout the world is complicated by regional genotype/phenotype differences. This study showed *N. gonorrhoeae* resistance genotypes/phenotypes and provided insight into a new antimicrobial agent, which could potentially be used in the control of *N. gonorrhoeae* infection.

The treatment of *N. gonorrhoeae* infections is limited because of increased antibiotic-resistant strains. As this represents a serious public health problem, it is important to develop new

bactericides. In the search for new compounds with biological activity curcumin and derivatives therefore have attracted the interest of scientists.

There were several researchers who investigated the anti-microbial activity of curcumin and its derivatives *in vitro* [13, 14]. The results showed that curcumin may be an alternative antimicrobial agent against fetal bacterial infections. Based on the molecular structure of curcumin, containing two carbonyl groups, Zhang et al. used aniline, 4-methyl aniline, and benzene nitrification as hydrazines, and 2,4-difloоronitrohenzene hydrazine curcumin as the raw material to synthesize four kinds of Schiff base curcumin stability derivatives, and the antimicrobial activity analysis *in vitro* revealed that four curcumin derivatives had good antibacterial activity [15].

In our experiments with curcumin using eight strains of *N. gonorrhoeae*, bacteriostatic annulus diameters of 6 mm were recorded (the diameter of the disc), indicating no bacteriostatic activity. However, this result was different to the result obtained from the cells [16].

On testing FM0817 curcumin against twenty-two strains of *N. gonorrhoeae*, the bacteriostatic annulus diameters showed no obvious differences in size (33-37 mm on average), also as the FM04 (8-11 mm on average). The mechanism of FM0817 and FM04 and the *N. gonorrhoeae* drug-resistance genotypes/phenotypes may not be related, and, furthermore, our analysis of drug-resistance strains and the genotypes/phenotypes of drug resistance may not be comprehensive. Therefore, further analysis of different resistance genotypes and drug-resistance strains of different phenotypes with respect to FM04 and FM0817 antimicrobial susceptibility is required. However, the FM0817 derivative of curcumin showed larger bacteriostatic annulus diameters (average 35 mm), showing antibacterial activity *in vitro*. However, the diameters of FM04 were small (8-11 mm). Because of the small number of isolates examined in the present study, further research is required to fully elucidate the antimicrobial properties and potential applications of the FM04 and FM0817 derivatives.

Disclosure of conflict of interest

None.

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