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Antimycoplasmal activities of (S)-(-)-9-fluoro-2,3-dihydro-3-methyl-10-[4-(2-pyridyl)-1-piperazinyl]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (YH-6) in comparison with other antibiotics *in vitro*[1]

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KEY WORDS *Mycoplasma*; *Ureaplasma*; erythromycin; leucomycins; tetracycline; josamycin; tylosin; microbial sensitivity tests; microbial drug resistence

ABSTRACT

AIM: To determine the susceptibilities of *Mycoplasma* and *Ureaplasma* to (S)-(-)-9-fluoro-2,3-dihydro-3-methyl-10-[4-(2-pyridyl)-1-piperazinyl]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (YH-6) and to compare it with those referential quinolones, macrolides, and tetracyclines. **METHODS**: The minimum inhibitory concentration (MIC) were determined by microdilution method *in vitro*. **RESULTS**: The MIC of YH-6 for *Ureaplasma urealyticum* (*Uu*; 250 $\mu g \cdot L^{-1}$), *Mycoplasma hominis* (*Mh*; 500 $\mu g \cdot L^{-1}$), *M orale* (*Mo*; 125 $\mu g \cdot L^{-1}$) and *M salivarium* (*Ms*; 125 $\mu g \cdot L^{-1}$) were closely similar to those of macrolides (erythromycin and leucomycin) and were 2 - 8 folds greater than those of ofloxacin (Ofl). *Uu* and *Mh* easily induced resistance to erythromycin and tetracycline. They did not easily form resistance to quinolone (YH-6, Ofl), josamycin and tylosin. Tetracycline-resistance (Tc^r) or erythromycin-resistance (EM^r) strains of *Uu* (or *Mh*) had cross-resistance to erythromycin or tetracycline. However, they had no cross-resistance to quinolone, josamycin and tylosin. **CONCLUSION**: YH-6 was a highly active quinolone against *Mycoplasma*, but could hardly induce resistance to *Uu*. EM^r- or Tc^r- strains of *Uu* (or *Mh*) had no cross-resistance to YH-6.

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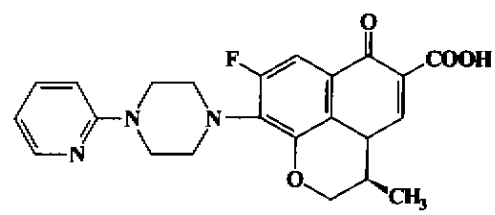
INTRODUCTION

Ureaplasma urealyticum (*Uu*) and *Mycoplasma hominis* (*Mh*) are important causes of nongonococcal ureathritis (NGU), *M orale* (*Mo*) and *M salivarium* (*Ms*) are generally infectious in culture of cell and biological conductions. Macrolide and tetracycline antibiotics have been widely used for chemotherapy against *Uu* and *Mh* infections. At the same time, the reported incidence of mutants resistant to erythromycin and tetracycline from patients treated with or without the antibiotics have increased[1,2]. Considerable research has led to the development of newer quinolones that not only are active against gram-negative organisms but also have high levels of activity against gram-positive organisms[3]. (S)-(-)-9-Fluoro-2,3-dihydro-3-methyl-10-[4-(2-pyridyl)-1-piperazinyl]-7-oxo-7H-pyrido[1,2,3-de][1,4] benzoxazine-6-carboxylic acid (YH-6) was an analog of levofloxacin (LVFX) which was synthesized by Dr YANG Yu-She[4]. So the *in vitro* susceptibilities of *Uu*, *Mh*, *Mo*, and *Ms* to YH-6 was studied, and compared with older quinolones, including NA, NFLX, Cp, Ofl, macrolides and tetracyclines as references.



YH-6

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MATERIALS AND METHODS

Mycoplasma The prototypic strains of *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mycoplasma orale*, and *Mycoplasma salivarium* were supplied by Prof ZHAO Ji-Wen (Railway Medical University of Nanjing, China).

Media The Mycoplasma media contain 1:1 beef heart infusion broth, 10 % unheated "agamma" horse serum, 10 % fresh yeast extract, 0.5 % NaCl, 3 % polypeptide, 0.15 % KH_2PO_4, and 0.002 % phenol red indicator. When used for *Mh*, *Mo*, and *Ms*, H broth at pH 7.0 – 7.2 contained 0.1 % *L*-arginine hydrochloride. For *Uu*, U broth at pH 5.5 – 6.5 contained 0.1 % urea.

Antimicrobial agents used were YH-6, nalidic acid (NA, Sigma Chemical Co, St Louis, USA), norfloxacin (NFLX) and ofloxacin (Ofl, Kunshan Pharmaceutical Co, China), ciprofloxacin (Cp, Shanghai Sunve Pharmaceutical Co, China), erythromycin (EM, Zhenjiang Pharmacetical Co, China), josamycin (Jos, Yamanouchi, Tokyo, Japan), leucomycin (LM, Wenzhou No 2 Pharmaceutical Co, China), tylosin (Ts) and tetracycline (Tc, National Control Institute of Veterinary Bioproducts and Pharmaceuticals, China), doxycycline (Dox, Shanghai No 5 Pharmaceutical Co, China).

The powders were dissolved in *N*, *N*-dimethylformamide (DMF, ≤5 %) and methanol (≤2.5 %), and diluted with distilled water to prepare solutions containing antibiotics 1.6 $g \cdot L^{-1}$. Stock solutions of antimicrobial agents were stored at – 20 ℃ and used within one month.

Susceptibility testing Tests were performed in Methyl-methacrylate "microtiter" plates, 0.1 mL of mycoplasmal suspension was added by pipette to 0.1 mL of dilution of antibiotic. The color changing units (CCU) per mL was 5×10^4, positive and negative control were included[5]. A CCU was the minimum inoculum required to produce growth as indicated by a color change in the phenol red indicator. The plates, sealed with plate sealers, were incubated at 37 ℃ and examined at 48 – 72 h for color change observed by naked eyes. When the color of the medium of the drug-free control changed from yellow to red, the minimal concentration of drug preventing the color change was defined as the MIC.

Development of resistance to antibiotics Both *Uu* and *Mh* were tested as follows: mycoplasmal suspension of 10^7 CCU $\cdot L^{-1}$ 0.1 mL was introduced into 0.9 mL of media, the initial concentration of antibiotics was of 0.5 MIC. The tube was incubated at 37 ℃ (*Uu* incubated for 16 – 18 h, *Mh* cultured for 36 – 48 h), and 0.1 mL of the resulting culture was used as inoculum for the next tube, in which the antibiotics concentration was increased by 25 %, This procedure was repeated 8 – 10 times, and the subcultures were tested for their resistance to antibiotics, as described before, if strains' MIC were enhanced 4 times, they were considered as resistant mutant[6]. At the same time, cross-resistance of *Uu* or *Mh* was assayed.

RESULTS

Susceptibility of *Mycoplasma* NA (MIC ≥8000 $\mu g \cdot L^{-1}$) was not potent against all *Mycoplasma* tested. Ofl has two folds greater potency than Cp, but inferior to YH-6, YH-6 for *Uu* (250 $\mu g \cdot L^{-1}$), *Mh* (500 $\mu g \cdot L^{-1}$) and *Mo*, *Ms* (125 $\mu g \cdot L^{-1}$) were comparable or superior to those of macrolide (EM) and had 2 – 8 folds greater potency than those of Ofl. Macrolides and tetracyclines were very potent for most *Mycoplasma*. Particularly, josamycin (MIC: 125 $\mu g \cdot L^{-1}$ for *Uu* and *Mh*; 7.8 $\mu g \cdot L^{-1}$ for *Mo*; 15.6 $\mu g \cdot L^{-1}$ for *Ms*) and doxycline (MIC: 31.25 $\mu g \cdot L^{-1}$ for *Uu*; 62.5 $\mu g \cdot L^{-1}$ for *Mh* and *Mo*; 125 $\mu g \cdot L^{-1}$ for *Ms*) were the most active in macrolides and tetracyclines, respectively (Tab 1).

Development of tetracycline-resistance (Tc^r) or erythromycin-resistance (EM^r) strains of *Uu* and *Mh* After *Uu* and *Mh* were induced 10 times by EM, their MIC was increased as much as 16 and 312-folds respectively; after *Uu* and *Mh* were induced 10 times by Tc, their MIC as much as 8 and 16-folds respectively. Quinolones (YH-6 and Ofl), Jos and Tyl did not easily induce resistance to *Uu* and *Mh* (Tab 2).

Cross-resistance of EM^r or Tc^r of *Uu* and *Mh* The MIC of EM^r strain of *Uu* (or *Mh*) was increased 4 (or 16 – 32) folds than its parent strain, The MIC of Tc^r strain of *Uu* (or *Mh*) was 8 (or 512) folds than its parent strain, furthermore, EM^r or Tc^r strains of *Uu* (or *Mh*) had cross-resistance to Tc or

Tab 1. Susceptibilites of *U urealyticum*, *M hominis*, *M orale*, and *M salivarium* to the YH-6 and other antibiotics. $n = 3$ independent experiments.

Antibiotics	MIC/$\mu g \cdot L^{-1}$ *Uu*	*Mh*	*Mo*	*Ms*
Quinolones				
NA	8 000	8 000	8 000	8 000
NFLX	8 000	4 000	8 000	4 000
Cp	8 000	2 000	1 000	1 000
Ofl	2 000	1 000	500	500
YH-6	250	500	125	125
Macrolides				
EM	250	250	250	250
LM	250	250	250	250
Jos	125	125	7.8	15.6
Ts	500	500	500	250
Tetracyclines				
Tc	500	125	500	500
Dox	31.25	62.5	62.5	125

Tab 2. Development of resistance to YH-6, Ofl, EM, Jos, Ts, and Tc in *Ureaplasma urealyticum* (*Uu*) and *Mycoplasma hominis* (*Mh*). $n = 3$ independent experiments.

Compound	MIC in *Uu*/$\mu g \cdot L^{-1}$ generation 1st	5th	7th	10th	MIC in *Mh*/$\mu g \cdot L^{-1}$ generation 1st	5th	7th	10th
YH-6	250	250	250	500	500	4 000	4 000	4 000
Ofl	2 000	4 000	4 000	4 000	1 000	2 000	4 000	8 000
EM	250	2 000	2 000	4 000	250	64 000	64 000	128 000
Jos	125	125	250	250	125	200	500	500
Ts	500	1 000	1 000	1 000	500	500	1 000	2 000
Tc	500	2 000	2 000	4 000	125	200	1 000	2 000

EM, however, they had not cross-resistance to Jos, Ts of 16-membered macrolides and quinolones (YH-6, Ofl) (Tab 3).

Tab 3. Susceptibility of parent, Tc^r or EM^r strain of *Uu*, *Mh* to other drugs. $n = 3$ independent experiments.

Compound	MIC in *Uu*/$\mu g \cdot L^{-1}$ parent	EM^r	Tc^r	MIC in *Mh*/$\mu g \cdot L^{-1}$ parent	EM^r	Tc^r
YH-6	250	250	500	500	500	500
Ofl	2 000	4 000	2 000	1 000	1 000	1 000
Jos	125	250	250	125	125	125
Ts	500	1 000	1 000	500	500	500
EM	250	4 000	2 000	250	64 000	128 000
Tc	500	2 000	4 000	125	2 000	2 000

DISCUSSION

Erythromycin of 14-membered macrolides has been used extensively for the treatment of infections of the upper and lower respiratory tract, genital tract, skin and soft tissue caused by susceptible bacterial species which include: *M pneumoniae* (*Mp*), *U urealyticum*, *Streptococcus spp*, *Staphylococcus spp*, as well as others. However, *Mh* is always resistant, and resistant-strains of *Uu* are now being reported with increasing frequency[1,2]. Erythromycin's MIC range was $\geq 32\,000\ \mu g \cdot L^{-1}$ for *Mh*, its MIC_{90} was 2000 $\mu g \cdot L^{-1}$ for *Uu* in Flemingham's papers[7]. Strains are resistant as a result of modification of the 50 S ribosome, which is the target site for the antibacterial activity of the macrolides, by a methylating enzyme, however, the 16-membered macrolides are poor inducers of the methylating enzyme, they are better absorbed and have a higher peak level in serum[7]. Therefore, the 16-membered macrolides (Jos, LM) are still better drugs in the treatment of Mycoplasmal infection. Tylosin is not only stable in media, but also its cytotoxic concentration is 300 folds of its MIC, while, tetracycline's cytotoxic concentration is only 17.5 folds of its MIC. So, tylosin is better drug in the treatment and prevention of *Mycoplasma* in cell culture and biological products.

The tetracyclines antibiotics including tetracycline, doxycycline and minocycline were often used in the treatment of NGU. Recently, the high-level Tc^r isolates *Uu*, *Mh*, and *Mp* are increasing. Seven percent *Uu* was resistant in Davis' papers[8]. Magathaes reported that 30 % *Uu* was resistant[9]. Tc^r determinant (tetM) is broad through a variety of transposon- and plasmid-mediated mechanisms[10]. High level of EM^r has been correlated with tetracycline-resistance[1]. These lead to therapeutic problems. Koutsky *et al* in 1983 concluded that "tetracycline could no longer be considered the drug of choice for treatment of mycoplasmal infections"[11].

Clinical failures have occurred when ciprofloxacin or ofloxacin has been used to treat respiratory tract bacterial infections or urinary tract mycoplasmal infections. Resistance monitoring of the clinical isolates and mutant strains has important significance in clinical drug treatment and new agents study. It is clear that the clinical judgement as to which new

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fluroquinolone to be used in the treatment of microoganism infection will be based upon several factors, including the provalence of fluoroquinolone-resistant bacteria, pharmacokinetics, toxicity, adverse reactions, and patient type. New quinolone (YH-6) has been found effective in low concentration against tetracycline-sensitive and Tc^r or erythromycin-sensitive and EM^r strains of *Uu* and *Mh*. Because of the different mechanism of action its bacteriocidal effect should be examined against Tc^r and EM^r isolates. The findings suggested that this compound (YH-6) has bright promise for treatment of mycoplasmal infections if appropriate pharmacokinetic and safety parameters can be established.

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(S)-(-)-9-氟-2,3-二氢-3-甲基-10-[4-(2-吡啶)-1-基哌嗪]-7-氧代-7H-吡啶[1,2,3-de][1,4]苯并噁嗪-6-羧酸(YH-6)与其它抗微生物药体外抗支原体活性的比较[1]

R978.15

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关键词 支原体; 尿素支原体; 红霉素; 柱晶白霉素类; 四环素; 交沙霉素; 泰洛星; 微生物敏感试验; 微生物抗药性

目的: 测定新喹诺酮 YH-6 对支原体的抑制活性并与其它抗微生物药剂作比较. **方法**: MIC 的测定采用微量稀释法. **结果**: YH-6 对解脲支原体(*Uu*), 人型支原体(*Mh*), 口腔支原体(*Mo*), 唾液支原体(*Ms*)的 MIC 分别为 250 $\mu g \cdot L^{-1}$, 500 $\mu g \cdot L^{-1}$, 125 $\mu g \cdot L^{-1}$, 125 $\mu g \cdot L^{-1}$, 它的抑制活性与红霉素, 柱晶白霉素相当, 是氧氟沙星的 2-8 倍. *Uu*, *Mh* 对 YH-6 及交沙霉素和泰洛星不易产生诱导耐药性, 而对红霉素和四环素易产生诱导耐药性和多重耐药性, 而对 YH-6, 交沙霉素和泰洛星无多重耐药性. **结论**: YH-6 对支原体有很强的抑制活性, 且不易形成诱导耐药性. *Uu* 和 *Mh* 的红霉素或四环素抗性菌株对 YH-6 无多重耐药性.

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