In Vitro Effects of Spectinomycin and Ceftriaxone Alone or in Combination with Other Antibiotics against *Chlamydia trachomatis*

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The in vitro effects of spectinomycin and ceftriaxone, alone or in combination with erythromycin, ofloxacin, and doxycycline, against *Chlamydia trachomatis* were investigated by the checkerboard method and compared by Ridit (reference identical unit) analysis. A combination of spectinomycin with erythromycin or doxycycline was found to be more effective than that of ceftriaxone.

Patients with genital chlamydial infection are often coinfecting with *Neisseria gonorrhoeae*. Many studies suggested the coinfection rate is between 20 and ~40% (17). Among the antimicrobials routinely used in the treatment of genital gonococcal infection, there are rarely effective agents that are also effective in a single dose against *Chlamydia trachomatis* (6, 7). The Centers for Disease Control and Prevention (CDC) recommends that treatment of gonococcal infection should be accompanied by treatment for genital chlamydial infection (2–5). Although the cost-effectiveness of this dual therapy has been evaluated (9, 14), little is known about the in vitro effects of interactions between the drugs used. Our aim was to investigate the in vitro effects of spectinomycin and ceftriaxone, which are commonly used in the treatment of gonococcal infection, alone or in combination with erythromycin, ofloxacin, or doxycycline, respectively, against *C. trachomatis* by a microdilution checkerboard assay.

A total of 10 *C. trachomatis* strains were obtained as fresh isolates from endocervical or urethral swabs from patients with urogenital symptoms at our sexually transmitted disease clinic. The isolates were typed by detection of restriction fragment length polymorphism in the gene encoding the major outer membrane protein of *C. trachomatis* by the method of Frost et al. (13). They consisted of serovars E ($n = 4$) and F ($n = 6$). Two reference strains, E-BOUR (ATCC VR-348B) and L3 (CDC), were used as quality controls. Antimicrobial agents were all obtained as powders from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China, and solubilized according to the manufacturer's instructions.

MICs were determined by the method described previously (8, 18, 19). Each test was performed in triplicate. Briefly, chlamydia strains (4×10^4 inclusion-forming units/ml) were inoc-

ulated onto confluent monolayers of McCoy cells (1×10^5 to $\sim 2 \times 10^5$ cells/well) in 96-well microtiter plates (Linbro, Flow Laboratories) with growth medium (1% fetal calf serum in RPMI 1640). After the inoculate had been centrifuged at $1,250 \times g$ for 1 h at 30°C, the supernatant was replaced with maintenance medium (growth medium supplemented with 1 μ g of cycloheximide per ml and 0.35% glucose) containing serially twofold-diluted antimicrobials. After incubation at 35°C for 48 h, the cultures in plates were fixed with acetone, stained with a fluorescein isothiocyanate (FITC)-labeled rabbit antibody specific to elementary bodies of *C. trachomatis* (Virostat, Portland, Maine), and observed under a fluorescence microscope. The MIC was defined as the lowest drug concentration at which no inclusion body was seen.

The in vitro effect of antimicrobial combination was evaluated by a two-dimensional (8-by-8) checkerboard microdilution technique. The procedures were similar to the determination of the MIC. The concentrations tested ranged from 4 to 1/16 times the MICs. The fractional inhibitory concentration (FIC) index for combinations was calculated as follows (11):

$$\text{FIC index} = \text{FIC}_A + \text{FIC}_B = \frac{(A)}{(\text{MIC}_A)} + \frac{(B)}{(\text{MIC}_B)}$$

(A) is the concentration of drug A in a well which is the lowest inhibitory concentration for *C. trachomatis* in its row or column. (MIC_A) is the MIC of drug A for *C. trachomatis*. FIC_A , the fractional inhibitory concentration of drug A, is derived from dividing (A) by (MIC_A). (B), (MIC_B), and FIC_B are defined in the same fashion for drug B. The interaction is defined as synergistic if the FIC index is ≤ 0.5 , antagonistic if > 4.0 , and indifferent if between 0.5 and 4.0. Each isolate for every combination was tested in triplicate. For a given isolate with a given combination, if synergism (or indifference or antagonism) was observed in two or three runs of the triplicate, synergism (or indifference or antagonism) was reported.

The effects of different combinations were compared by reference identical unit (Ridit) analysis. Ridit, introduced by Bross in 1958, is a sensitive statistical method to analyze ordi-

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TABLE 1. Comparison of the three combinations with spectinomycin or ceftriaxone

Combination ^a	Result by:				<i>P</i> ^b
	Synergism (FIC ≤ 0.5)	Indifference (0.5 < FIC ≤ 4)	Antagonism (FIC > 4)	Ridit	
SPT-ERY	5	7	0	0.55	0.08
SPT-OFX	0	12	0	0.35	
SPT-DOX	6	6	0	0.60	
CRO-ERY	0	12	0	0.5	0.79
CRO-OFX	1	11	0	0.54	
CRO-DOX	0	11	1	0.46	

^a SPT, spectinomycin; ERY, erythromycin; OFX, ofloxacin; DOX, doxycycline; CRO, ceftriaxone.

^b *P* for comparisons of three combinations (e.g., SPT-ERY, SPT-OFX, and SPT-DOX).

nal data. The Ridit score does not require a normal distribution and preserves differences present in the (ordinal) data. Ridit analysis illustrates the relative position of the score of a particular group against an identified distribution of a reference group (20). The analysis was carried out by the calculation function of Excel Table.

In each batch of microdilution tests, the MICs of the five antimicrobial agents for strains VR-348B and L3 all fell within the ranges previously observed by other investigators using the same method (10, 15, 22). The ranges of MICs of spectinomycin, ceftriaxone, erythromycin, ofloxacin, and doxycycline were 128 to 256, 10 to 20, 0.06 to 0.5, 0.25 to 1, and 0.015 to ~0.125 µg/ml, respectively, in our study.

The in vitro effects and comparisons of combinations are shown in Tables 1 and 2. Indifference was observed in most of the combinations. When the combination of spectinomycin with erythromycin, ofloxacin, or doxycycline was compared to that with ceftriaxone, respectively, both combinations of spectinomycin with erythromycin (*P* = 0.014) and doxycycline (*P* = 0.005) were more synergistic than the combinations of ceftriaxone with the correspondent antibiotics. The combinations of ofloxacin with spectinomycin and ceftriaxone were of no significant difference (*P* = 0.32).

Our results are similar to those observed with other bacteria

TABLE 2. Comparison between spectinomycin and ceftriaxone in the three combinations

Combination ^a	Result by:				<i>P</i> ^b
	Synergism (FIC ≤ 0.5)	Indifference (0.5 < FIC ≤ 4)	Antagonism (FIC > 4)	Ridit	
ERY-SPT	5	7	0	0.60	0.014
ERY-CRO	0	12	0	0.39	
OFX-SPT	0	12	0	0.48	0.32
OFX-CRO	1	11	0	0.52	
DOX-SPT	6	6	0	0.63	0.005
DOX-CRO	0	11	1	0.36	

^a ERY, erythromycin; SPT, spectinomycin; CRO, ceftriaxone; OFX, ofloxacin; DOX, doxycycline.

^b *P* for comparisons of two combinations (e.g., ERY-SPT and ERY-CRO).

in previous studies. Burrows et al. (1) observed that the combination of erythromycin with spectinomycin was synergistic or additive against 27 of 33 *Pasteurella haemolytica* isolates. Flatz et al. (12) found synergism in penicillin-resistant pneumococci for the combination of ceftriaxone and levofloxacin, with a FIC value of 0.25. Mayer et al. (16) found synergism in 4 of 18 clinical isolates of *Pseudomonas* spp. for the combination of ceftriaxone with ofloxacin. Shishkov et al. (21) showed that spectinomycin-tetracycline was a most effective combination against *Mycoplasma gallisepticum*. Yet, there is an issue in our study which may be worthwhile to mention here. The interpretation of the FIC index was described to have different definitions, which may lead to inconsistent conclusions (23). The ineffectiveness of combinations of ceftriaxone in our study may be more clear (data are not shown) if the definition for antagonism is, traditionally, a FIC index of no less than 2 (11).

Most of the synergism between spectinomycin and doxycycline or spectinomycin and erythromycin occurred at a sub-MIC or even much lower concentration of spectinomycin (e.g., 32 µg/ml). The average reduction of MICs was two- or four-fold. Though the synergism was very weak, our study still recommended spectinomycin rather than ceftriaxone for this dual therapy because of the significant difference between the combinations of spectinomycin and ceftriaxone. In other words, when ceftriaxone is prescribed in the therapy, the interval between or the sequence of the administration of ceftriaxone and erythromycin or doxycycline may be prolonged or shifted to avoid possible antagonism and to achieve the best effect from combination therapy. In addition, in vivo study is required to confirm the results and the clinical significance of the conclusion in this study. Besides, more effective combinations need to be explored for this dual therapy.

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