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The antimicrobial susceptibility of *Chlamydia trachomatis* in cell culture

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SUMMARY The action of 22 antimicrobial agents against the SA_2f strain of *Chlamydia trachomatis* has been studied by the use of a simple cell culture technique. Tests for bactericidal activity were undertaken with some of the agents, and latency of infection was demonstrated. The susceptibilities of 10 clinical strains of *C. trachomatis* and of SA_2f to oxytetracyline, erythromycin, and spectinomycin in cell culture were found to be identical.

Introduction

The importance of *Chlamydia trachomatis* as a pathogen in the human genital tract is now well recognised, but the study of the action of antimicrobial agents against *Chlamydia in vitro*, an essential preliminary to rational treatment, was long hampered by the lack of a simple and reliable experimental system. Early work with embryonated chicken egg yolk sacs and with animal models (Werner, 1961) yielded valuable data, but the methods were slow and cumbersome. Recently, however, advances in cell culture systems (Gordon and Quan, 1965; Wentworth and Alexander, 1974) have made the *in vitro* testing of antimicrobial drugs against *Chlamydia* much easier. We have described a technique with which eight drugs were tested against a laboratory-maintained strain of *C. trachomatis* (SA_2f) in McCoy cell cultures (Ridgway *et al.*, 1976), and Kuo *et al.* (1977) investigated the action of seven drugs against the 15 known immunotypes of *C. trachomatis* in HeLa 229 cell cultures.

In the experiments reported here we have extended our earlier study in three directions: (1) We have estimated the minimum inhibitory concentration (MIC) of 22 antimicrobial agents against SA_2f. As before, MIC is defined as the lowest concentration of drug preventing the appearance of any chlamydial inclusions in the cell monolayers. (2) The minimum cidal concentration (MCC) of eight agents against SA_2f was estimated. MCC is defined as the minimum concentration of drug required to prevent further infection of the cell monolayers, even after multiple passage. *C. trachomatis* infections exhibit latency (Hanna *et al.*, 1968), so for any particular drug the MIC and MCC may not necessarily be the same. (3) To see whether experience derived from studies with SA_2f can be applied to clinical isolates of *C. trachomatis*, the MIC of various antimicrobial agents against SA_2f and against 10 freshly isolated strains of *Chlamydia* were compared.

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Materials and methods

The organism used for most of this work was the SA_2f strain of *C. trachomatis*, but some experiments were also done with low passage isolates from patients with non-gonococcal urethritis or cervicitis.

DETERMINATION OF MIC

The method has been described elsewhere (Ridgway *et al.*, 1976). Briefly, doubling dilutions of the antimicrobial agent were prepared in agent-free *Chlamydia* growth medium. One millilitre of each dilution was added to a flat-bottomed plastic tube containing a coverslip monolayer of 5-iodo-2-deoxyuridine (IUDR) treated McCoy cells. A suspension of *Chlamydia* containing approximately 200 inclusion-forming units per ml was added to each tube. After centrifugation at 3000 *g* for an hour, the tubes were incubated for between 38 and 40 hours at 35°C. The coverslips were removed and the cell monolayer was fixed with methanol and stained either with Giemsa for examination of autofluorescence using darkground illumination, or with iodine for examination of stained inclusions by brightfield illumination. Both methods of staining

were used in case some of the antimicrobial agents affected only one, as is known to happen, for example, with penicillins which inhibit glycogen production with the consequent formation of inclusions which stain poorly with iodine (Jawetz, 1969). The MIC was taken as the lowest concentration containing no inclusions with either staining method.

DETERMINATION OF MCC

Four tubes containing IUDR-treated McCoy cells were prepared for each dilution of antimicrobial drug, and inoculated and incubated as above. After incubation, the coverslips from two tubes were fixed and stained, one with Giemsa and the other with iodine. The medium was removed from the remaining two tubes and replaced with 2 ml of medium free from antimicrobial drugs. The cells were rubbed off the coverslip with a sterile rubber-tipped glass rod, resuspended in the medium, and used to inoculate two further tubes containing fresh coverslip monolayers of IUDR-treated McCoy cells. Thus the contents of the two tubes were passaged into four tubes. After centrifugation, tubes were incubated in the usual manner. This passage procedure was repeated as required, and the MCC was taken as the lowest concentration of antimicrobial agent preventing demonstrable inclusions after 10 passages.

Strict precautions were taken to prevent cross-contamination with *Chlamydia*. No other work with this organism was undertaken while passage experiments were being performed, safety cabinets were always used, and work proceeded from higher to lower concentrations of antimicrobial agent.

Results

MIC

Table 1 shows the MIC of various antimicrobial agents to the SA_2f strain. For all drugs except penicillins, cephalosporins, and folic acid antagonists (sulphamethoxazole and trimethoprim) the end point was sharp, and was the same whether Giemsa or iodine staining was used. Penicillins, cephalosporins, and the folic acid antagonists produced abnormal forms which often persisted throughout the dilutions tested. The MIC of these drugs was taken to be the lowest concentration which showed no autofluorescent inclusions on darkground examination; this coincided with the predominance of the abnormal forms over the normal inclusions. To determine whether the abnormal inclusions were viable, multiple passage was performed with some agents.

MCC

A typical result of a test for the MCC of an antimicrobial agent is shown for sulphamethoxazole in Table 2. In this case, no tubes yielded inclusions after the sixth passage. It will be seen that several 'blind' passages were necessary before inclusions could be seen by light microscopy in tubes containing concentrations below the MCC. The results for all the drugs tested by passage are shown in Table 3. The number of passages required before no further tubes yielded inclusions varied between drugs, but was constant for each.

CLINICAL ISOLATES

The action of oxytetracycline, erythromycin, and spectinomycin was assessed against 10 strains of *C. trachomatis* recovered from patients with NGU or cervicitis, using SA_2f as a control. The results are shown in Table 4. There was no marked difference between the results obtained with these isolates and with SA_2f.

Discussion

SA_2f is immunologically identical with the LGV II serotype (Wang and Grayston, 1970), which cross-reacts with Groups B and ED, immunotypes

Table 1 *Minimal inhibitory concentrations (MIC) of various antimicrobial agents against* Chlamydia trachomatis *(SA_2f) in cell culture*

High activity		*Medium activity*		*Low activity*	
Antimicrobial	*MIC (μg/ml)*	*Antimicrobial*	*MIC (μg/ml)*	*Antimicrobial*	*MIC (μg/ml)*
Rifampicin	0·007	Ampicillin	0·25	Spectinomycin	64
Doxycycline	0·03	Mecillinam	0·25	Oxolinic acid	128
Minocycline	0·03	Penicillin	1·0	Trimethoprim	128
Oxytetracycline	0·06	Clindamycin	1·0	Cefuroxime	256
Erythromycin (base and lactobionate)	0·06	Rifamide	1·0	Lincomycin	512
		Amoxycillin	2·0	Vancomycin	>256
		Cephaloridine	2·0	Metronidazole	>256
		Sulphamethoxazole	4·0	Gentamicin	>512
		Chloramphenicol	4·0		

Table 2 *Recovery of* Chlamydia trachomatis (*SA_2f*) *after initial incubation with sulphamethoxazole*

Concentration of sulphamethoxazole (μg/ml)	*Passage number* 0	1	2	3	4	5	6	7	9	9	10
1·0	+	−	+/−	+/−	+	ND	ND	ND	ND	ND	ND
2·0	+	−	−	−	+	ND	ND	ND	ND	ND	ND
4·0	(−)	−	−	−	−	+	ND	ND	ND	ND	ND
8·0	(−)	−	−	−	−	−	+	ND	ND	ND	ND
16.0	(−)	−	−	−	−	−	+	ND	ND	ND	ND
32·0	(−)	−	−	−	−	−	−	−	−	−	−

\+ Giemsa-stained autofluorescent inclusions
(−) Autofluorescence absent; tiny abnormal inclusions present
− Inclusions not seen by light microscopy
ND Not done
MIC and MCC of sulphamethoxazole in this experiment were 4 μg/ml and 32 μg/ml respectively

Table 3 *Relationship between minimum inhibitory concentration and minimum cidal concentration for various antimicrobial agents against* Chlamydia trachomatis (*SA_2f*)

Antimicrobial agent	*Minimum inhibitory concentration* (μg/ml)	*Minimum cidal concentration* (μg/ml)	*No. of passages required for cidal end-point*
Oxytetracycline	0·06	0·25	2
Erythromycin	0·06	0·5	3
Mecillinam	0·25	>1·0	2
Cephaloridine	2·0	>32·0	3
Sulphamethoxazole	4·0	32·0	6
Spectinomycin	64	128	1
Trimethoprim	128	1024	3
Cefuroxime	256	>512	1

Table 4 *Minimum inhibitory concentrations of various antimicrobial agents to clinical isolates of* Chlamydia trachomatis *in cell culture*

Strain no.	*Serotype*	*Oxytetracycline* (μg/ml)	*Erythromycin* (μg/ml)	*Spectinomycin* (μg/ml)
1	E	0·03	0·06	32
2	DE	0·03	0·03	64
3	DE	0·06	0·03	32
4	G	0·06	0·12	64
5	DE	0·06	0·06	64
6	D	0·03	0·03	64
7	D	0·06	0·03	64
8	D	0·06	0·03	64
9	D	0·06	0·03	64
10	D	0·06	0·03	64
SA_2f	LGV II	0·06	0·06	64

commonly associated with human genital infections. In the present study the MICs of oxytetracycline, erythromycin, and streptomycin against 10 genital strains and SA_2f were closely similar. All but one of these clinical isolates were in the DE complex of immunotypes, reflecting their genital origin, but Kuo *et al.* (1977), who tested all the 15 known immunotypes of *C. trachomatis*, found only minor differences between their responses to tetracycline and erythromycin. Thus, SA_2f appears to be a suitable organism in evaluating the anti-chlamydial action of antimicrobial drugs for use in genital tract infections, and it also has the advantages of being readily available and safe to laboratory workers.

Rifampicin was the most active of the drugs tested, but it has not been evaluated clinically for the treatment of genital tract infections. The tetracyclines and erythromycin are both highly active against *Chlamydia*, as earlier work with laboratory models has shown (Jawetz, 1969). There was a marked difference between the MICs of lincomycin and clindamycin, between rifampicin and rifamide, and between cephaloridine and cefuroxime. It is possible that these differences are owing to variations in the cell-penetrating properties of the antibiotics. The activity of related antimicrobial agents evidently cannot be predicted from the results obtained for one member of the group.

With some drugs there was a considerable difference between the MIC and the MCC. This was particularly apparent with the cell wall inhibitors, mecillinam and cephaloridine, and with the folic acid antagonists, sulphamethoxazole and trimethoprim. It is well known that cell wall inhibitors, such as penicillin, promote the formation of abnormal chlamydial inclusions. Johnson and Hobson (1977) were unable to render these abnormal forms viable by replacing the penicillin-containing medium with penicillin-free medium, without passage. However, Kuo *et al.* (1977), using single passage, demonstrated large differences between MIC values for infectivity and for viability of both penicillin and ampicillin against strains of *C. trachomatis*. Our own studies with mecillinam indicate that these inclusions readily recover their infectivity on passage on to fresh cell monolayers in medium free from antimicrobial drugs, but that multiple passage may be necessary.

The data summarised in Table 3 indicate that after the action of some agents it may be impossible to see chlamydial inclusions by microscopical examination, although after passage normal infectious inclusions may reappear. These observations suggest that within the system described here some antimicrobial drugs induce latency. In ocular chlamydial infections latency is thought to be common (Hanna *et al.*, 1968), and it has been postulated that this is so for genital tract infections (Richmond *et al.*, 1972), but latency with *C. trachomatis* has not previously been demonstrated *in vitro*.

MIC estimations broadly indicate which compounds are likely to be effective in the treatment of genital chlamydial infection; however, the results should be interpreted with caution, as *in vitro* the cidal concentration may differ considerably from the inhibitory concentration. Furthermore, if latency can be induced by antimicrobial drugs *in vivo* as well as *in vitro*, the concept of cure of these infections may require re-evaluation, and longer periods of follow-up after treatment than are currently practised may be advisable.

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