Susceptibility of *Chlamydia trachomatis* to Excipients Commonly Used in Topical Microbicide Formulations

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Commonly used “inactive” pharmaceutical excipients were tested in a previously developed minimum cidal concentration assay to assess their ability to kill *Chlamydia trachomatis* topically. Sixteen excipients were evaluated in these studies under various conditions. A range of activities was found among the excipients that could be tested in our assay system.

Promising topical antimicrobial agents that are active against sexually transmitted infection pathogens have been identified (1, 10, 11), but these agents must be delivered in an acceptable dose and formulation such as a cream, gel, or foam. These drug delivery systems contain excipients that serve multiple functions, including the enhancement of product stability, efficacy, and acceptability to the patient. Excipients can be classified into a number of categories, including preservatives, solvents, antioxidants, and gelling agents. Although excipients are important components of topical microbicide products and are also known to possess antimicrobial activity (4, 12), their activity against *Chlamydia trachomatis* have not yet been determined. Here we use the previously described (1, 10, 11) minimal cidal concentration (MCC) assay, designed to test the direct action of microbicides against the extracellular, infectious *C. trachomatis* elementary bodies (EBs), to measure the inherent antichlamydial activity of potential excipients for formulation of active agents into topical microbicide products.

These studies used *C. trachomatis* serovar L2 (434/Bu). The bacterium was propagated in mouse McCoy fibroblasts (ATCC CRL 1696), purified on Renografin (6), and frozen at -70°C in a sucrose-phosphate-glutamate cryopreservative buffer (SPG) (8). The serotype of this strain was verified by a plate typing method (13). This strain was used because it is easier to grow and more sensitive to some topical microbicides than other strains (3).

Excipients evaluated in these studies represented four principal categories. The antioxidants evaluated were vitamin E (Spectrum, Gardena, Calif.), EDTA (Sigma, St. Louis, Mo.), and butylated hydroxyanisole (Spectrum). The preservatives tested were propylparaben (Spectrum), methylparaben (Spectrum), sodium benzoate (Spectrum), potassium benzoate (Spectrum), benzalkonium chloride (BAK; Sigma), benzoic acid (Spectrum), sorbic acid (Spectrum), and PEG 400 (Spectrum). The acidifying agents were lactic acid (Spectrum) and citric acid (Sigma). Gelling agents studied were three carrageenan products, Viscarin 328, Gelcarin 812, and Seaspen (FMC, Newark, Del.). These excipients were evaluated at

three concentrations (lower than the average, average, and higher than the average normal usage level in vaginal products) (9), two pH values (pH 5 and 7), and two time points (5 and 120 min). Most excipients were dissolved in SPG, while a few (sorbic acid, methylparaben, and benzoic acid) were solubilized in ethyl alcohol and then diluted in SPG. The concentration of ethyl alcohol used, however, was shown to have no activity against the chlamydial EBs (data not shown). Four of the excipients (propylparaben, vitamin E, Seaspen, and butylated hydroxyanisole) could not be tested in our assay system at any of the given concentrations due to low aqueous solubility. Five (sorbic acid, benzoic acid, Gelcarin 812, methylparaben, and Viscarin 328) could be solubilized only at the lowest concentrations tested. Lactic acid could be brought to the appropriate pH only at the lowest concentration without seriously affecting the assay conditions. Citric acid could be brought to the appropriate pH only at the lowest and average concentrations.

Because host cell health is crucial in chlamydial testing, we first measured the cytotoxicity of the excipients on McCoy cells by using Alamar blue (Biosource International Inc., Camarillo, Calif.). The goal was not to examine general excipient cytotoxicity but rather cytotoxicity in our *C. trachomatis* culture system. The MCC assay procedure was followed, except that the addition of *C. trachomatis* to the test compound was omitted and cytotoxicity was determined as described previously (10).

To carry out the MCC assays, excipients were diluted to the desired concentrations in SPG at the appropriate pH to bring the final pH to 5 or 7. Minor adjustments of pH were done with 1 M KH_2PO_4 or 1 M Na_2HPO_4 . A total of 10^6 *C. trachomatis* serovar L2 inclusion-forming units (IFU) in SPG at the same pH as that of the test compound were added to equal volumes of the diluted excipients and mixed well by pipetting. Four of the excipients (benzoic acid, citric acid, EDTA, and sorbic acid) were tested at both pH 5 and pH 7 with an inoculum that was diluted in SPG, pH 7.5. In these cases, when the inoculum was mixed in equal volumes with the excipient and adjusted to pH 5, the pH of the final mixture was an acidic value between 5 and 7.5. The standard MCC protocol was followed (10). *C. trachomatis* inclusions were counted in three fields for each of the three wells per excipient concentration, and inclusion counts for all nine fields were averaged. Average inclusion

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TABLE 1. Activities of excipients against *C. trachomatis*

Excipient	Concn (%)	% Inhibition of inclusion formation at:			
		pH 5, 5 min	pH 5, 120 min	pH 7, 5 min	pH 7, 120 min
BAK	0.0075	98.6	100.0	100.0	100.0
	0.0150	100.0	100.0	100.0	100.0
	0.030	100.0	100.0	100.0	100.0
PEG 400	1.00	17.6	35.8	16.5	32.0
	15.00	29.6	83.8	23.1	98.1
	30.00	40.3	100.0	35.1	100.0
Citric acid	0.575	2.7	1.2	10.5	11.1
	1.150	21.7	13.3	18.6	30.4
EDTA	0.005	11.7	17.5	0.3	27.9
	0.0525	7.8	28.7	10.4	47.0
	0.10	4.9	16.4	9.4	48.0
Potassium benzoate	0.250	-0.8	-26.0	0.0	14.8
	0.50	1.6	-12.5	10.2	10.0
	1.00	4.7	24.0	12.6	35.6
Sorbic acid	0.0625	16.2	16.9	11.1	18.4
	0.1250	23.0	25.1	19.0	48.5
Benzoic acid	0.0750	-2.2	3.3	5.6	2.9
	0.150	7.3	8.9	4.2	11.7
Gelcarin 812	0.3250	-8.8	-51.1	19.0	11.2
	0.50	21.3	-25.6	21.0	17.8
Lactic acid	0.034	20.5	-1.4	12.8	14.0
Methylparaben	0.07	12.1	-13.6	4.6	6.8
Sodium benzoate	0.25	4.7	-30.4	8.9	-4.6
	0.50	0.0	-22.6	15.6	4.2
	1.00	10.2	-35.7	5.0	19.9
Viscarin 328	0.15	17.6	40.9	14.1	10.9

counts were multiplied by a lens factor to determine the total number of IFU. Each excipient was tested once, and the percent inhibition of inclusion formation by the excipients was calculated using the following formula: percent killing = [(mean total number of IFU in organism control - mean total number of IFU in test)/(mean total number of IFU in organism control)] $\times$ 100. McCoy cells were used to determine the numbers of viable *C. trachomatis* EBs because they have been optimized for maximum chlamydial growth in our laboratory. These cells did not come in contact with the microbicide or excipient but were used only to grow *C. trachomatis* after exposure.

Using the MCC assay, we measured the ability of excipients to kill *C. trachomatis* EBs before they were able to infect the host cell (Table 1). All of the excipients evaluated had low cytotoxicities (less than 25%) to McCoy cells in the Alamar blue assay, indicating that if any chlamydial inhibition occurred, the compounds were acting directly on the EBs and were not acting indirectly through cytotoxicity to the McCoy cells in our culture system. Two of the excipients, BAK and PEG 400, had significant antichlamydial activities between 60 and 100% inhibition of inclusion formation. Four excipients

(citric acid, EDTA, potassium benzoate, and sorbic acid) showed moderate activities in the 20 to 60% range under the test conditions of pH 7 and 120 min of exposure. Six of the excipients (benzoic acid, Gelcarin 812, lactic acid, methylparaben, sodium benzoate, and Viscarin 328) had low activities under the test conditions of pH 7 and 120 min of exposure.

We included an acidic pH (pH 5) in this assay because it more closely mimics the pH of the normal human vagina, where the final product would be used. Some of the excipients (potassium benzoate, sodium benzoate, methylparaben, lactic acid, and Gelcarin 812) showed negative killing compared to the control at pH 5 after 120 min of exposure. This suggests that *C. trachomatis* EBs do not survive well in SPG at pH 5 and that the presence of the excipient protected the organism against the SPG at this low pH.

In general, the excipients had either significant activity (BAK and PEG 400), moderate activity (citric acid, EDTA, potassium benzoate, and sorbic acid), or no activity against *C. trachomatis*. There were no major differences in excipient activity at either pH 5 or pH 7, at the 5- and 120-min time points, and at the three concentrations, except in the case of PEG 400. All other excipients were generally equally active or inactive at the two time points, two pH values, and three concentrations. BAK was the most active excipient against *C. trachomatis* in these studies, with 100% inhibition at most concentrations, times, and pHs tested. These results are in agreement with those of Ito and Lyons (7), who also found killing of >99% of *C. trachomatis* EBs with 0.004% or more BAK, and with those of Belec et al. (2), who found killing of >99% of *C. trachomatis* EBs by 0.00125% BAK after 1 min of exposure. Herold et al. (5), however, found no chlamydial inhibition at a BAK concentration of 0.01%. Their results may be explained by the flow cytometric method used to measure the effect of BAK on the organism. The remaining excipients have not previously been tested for their topical activity against *C. trachomatis*; therefore, results to compare with our results are not available.

This is the first study examining the antimicrobial effects of excipients on *C. trachomatis*. These studies stress the importance of testing all components of putative topical microbicides for activity. This information will help when considering which ingredients to include in a topical microbicide formulation. In the presence of other compounds, the effects of some of these excipients may change. With this knowledge, however, one can more accurately assess the activity of the active ingredient against *C. trachomatis* in the final product.

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