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Effect of Hydrocortisone Succinate on Growth of *Chlamydia pneumoniae* In Vitro

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We examined the effect of hydrocortisone succinate on the growth of three isolates of *Chlamydia pneumoniae* in vitro. There was a significant increase in the number of inclusions seen in two of the *C. pneumoniae* strains in the presence of hydrocortisone. There was no significant increase in the number of inclusions with various concentrations of hydrocortisone over time. The addition of hydrocortisone did not affect the in vitro activities of azithromycin, erythromycin, and doxycycline against *C. pneumoniae*.

Airway inflammation is the most significant feature of asthma, leading to airway hyperreactivity as well as airway narrowing. Recent guidelines on the pharmacological treatment of asthma emphasize the importance of topical and/or systemic steroids (9). However, steroids are known to cause immunosuppression, and there has been concern about the effect of steroids on the clinical course of bacterial and viral infections associated with asthma.

Chlamydia pneumoniae is emerging as a potentially important pathogen in asthma. In 1991, Hahn et al. (5) described an association between serological evidence of acute *C. pneumoniae* infection and adult-onset asthma. Subsequently, we isolated *C. pneumoniae* from 13 (11%) of 118 children with acute episodes of wheezing (3). Seven of these children were being treated with inhaled and/or systemic steroids, and two remained culture positive for 3 to 5 months.

Previous studies (1) have demonstrated that steroids enhance the growth of *Chlamydia trachomatis* in vitro. To assess the effects of steroids on the infectivity of *C. pneumoniae*, we studied the growth of *C. pneumoniae* in HEp-2 cells in the presence of hydrocortisone succinate. We also examined the effect of hydrocortisone succinate on the activities of various antibiotics (erythromycin, doxycycline, and azithromycin) against *C. pneumoniae*.

MATERIALS AND METHODS

Chlamydiae. The strains of *C. pneumoniae* used in the study were TW-183 (Washington Research Foundation, Seattle) and two clinical isolates from Brooklyn, BAL-14 and T2023 (ATCC VR-1356).

Hydrocortisone succinate. A stock solution of hydrocortisone succinate (Upjohn, Kalamazoo, Mich.) was dissolved according to the manufacturer's instructions and was diluted with fresh overlay medium (Iscove's modified Dulbecco's medium [GIBCO] containing 1 μg of cycloheximide [Sigma Chemical Co., St. Louis, Mo.] per ml and 10% fetal calf serum) to final hydrocortisone succinate concentrations of 0.25, 1.0, and 3.0 μg/ml.

Culture of *C. pneumoniae*. The chlamydia were cultured in cycloheximide-treated HEp-2 cells grown in 96-well microtiter plates (11). HEp-2 cell monolayers were inoculated with 10^3 inclusion-forming units (IFU) of each strain of *C. pneumoniae* per ml in duplicate, fresh overlay medium containing the concentrations of hydrocortisone succinate described above was added, and the plates were incubated for 72 h. The plates were fixed and stained with fluorescein-conjugated antibody to the chlamydia lipopolysaccharide genus antigen (Pathfinder Chlamydia Culture Confirmation System; Kallestad Diagnostic, Chaska, Min.) for determination of the number of IFU. The numbers of inclusions per well were counted and then corrected for the dilution factors.

Drugs. Erythromycin (Lilly Pharmaceuticals, Indianapolis, Ind.), doxycycline (Sigma), and azithromycin (Pfizer, Groton, Conn.) were prepared in stock solutions of 1,280 μg/ml according to their potencies and the manufacturers' instructions. Serial twofold dilutions of all drugs were prepared on the day of use.

Effect of addition of hydrocortisone succinate at various time points after infection. HEp-2 cells were inoculated with 10^3 IFU strain of TW-183 per ml. Three concentrations of hydrocortisone succinate were added at different time points from 0 to 48 h (0, 3, 6, 9, 12, 24, and 48 h) after infection. The wells were fixed and stained 72 h later, and the numbers of IFU present in wells with different drug concentrations were counted at each time point.

Antimicrobial susceptibility testing. *C. pneumoniae* was cultured in HEp-2 cells grown in 96-well microtiter plates with inoculation of 0.2 ml of 10^3 IFU of strains TW-183, BAL-14, and T2023 per ml. The plates were centrifuged at $2,000 \times g$ for 1 h, and the media were then aspirated and overlaid with 0.2 ml of medium containing serial twofold dilutions of erythromycin, doxycycline, and azithromycin with and without 0.25, 1, and 3 μg of hydrocortisone succinate per ml. After incubation at 35°C for 72 h, the cultures were fixed and stained for inclusions with fluorescein-conjugated antibody to the lipopolysaccharide genus antigen. The MIC was the lowest antibiotic concentration at which no inclusions were seen. The minimal chlamydiacidal concentration (MCC) was determined by freezing the cultures at −70°C and then thawing them, passing the disrupted cell monolayers onto new cells, incubating them for 72 h, and then fixing and staining them as described above. The MCC was the lowest antibiotic concentration which resulted in no inclusions after passage.

Statistical methods. The comparison of the differences due to concentrations and passage as well as the interaction between concentration and passage were analyzed by analysis of variance. Subsequently, a post hoc comparison was performed by the least-significant-difference test.

RESULTS

Effect of hydrocortisone succinate on the growth of *C. pneumoniae* in HEp-2 cells. As indicated in Table 1, the numbers of

TABLE 1. Effect of hydrocortisone succinate on the growth of *C. pneumoniae* in HEp-2 cells[a]

Hydrocortisone succinate concn (μg/ml)	No. of IFU[b]		
	TW-183	BAL-14	T2023
0 (control)	1,235	814	2,784
0.25	2,884[c]	2,668[c]	3,592 (NS[d])
1	2,570[c]	1,692[c]	2,520 (NS)
3	2,020[c]	3,816[c]	2,920 (NS)

[a] After one passage.
[b] Each count is the mean of three wells.
[c] $P < 0.05$ compared with control.
[d] NS, no significant difference from mean for untreated controls.

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TABLE 2. Effect of addition of hydrocortisone succinate after infection of HEp-2 cells with TW-183

Time (h) after infection that HS[a] was added	No. of IFU in presence of HS at[b]:		
	0.25 μg/ml	1 μg/ml	3 μg/ml
0	3,576[c]	3,390[c]	3,354[c]
3	3,942[c]	3,048 (NS[d])	3,060 (NS)
6	3,306[c]	3,396[c]	3,348[c]
9	2,856 (NS)	3,246[c]	3,306[c]
12	3,048[c]	2,880 (NS)	3,186[c]
24	2,808 (NS)	2,784 (NS)	2,850 (NS)
48	2,970 (NS)	3,036 (NS)	2,982 (NS)
Control (no additional HS)	2,712	2,743	2,676

[a] HS, hydrocortisone succinate.
[b] Each count is the mean of two wells.
[c] $P < 0.05$ compared with control.
[d] NS, no significant difference from mean of controls.

inclusions of strains TW-183 and BAL-14 that grew in the presence of hydrocortisone succinate were significantly higher than the numbers of cells that grew without hydrocortisone succinate. The effect of hydrocortisone succinate did not seem to be dose related. There was no significant effect on the growth of strain T2023 when hydrocortisone succinate was added.

Effect of addition of hydrocortisone succinate at various times after inoculation of TW-183. The number of IFU after the addition of hydrocortisone succinate at different time points is presented in Table 2. The growth of strain TW-183 was significantly enhanced when hydrocortisone succinate was added 0 to 12 h after infection of the cells. The addition of hydrocortisone succinate after the first 24 h after inoculation did not have a significant effect on the growth of TW-183. The effect of hydrocortisone succinate was not dose dependent.

Antimicrobial susceptibility tests. As indicated in Table 3, the MICs and MCCs of erythromycin, doxycycline, and azithromycin for strains TW-183, BAL-14, and T2023 were not significantly different with or without hydrocortisone succinate.

DISCUSSION

C. pneumoniae appears to be capable of causing a wide range of respiratory tract illnesses, ranging from pharyngitis to pneumonia (4). We have also seen persistent *C. pneumoniae* infection after acute respiratory illness in several adult patients for periods of up to 11 months (6). Recent studies have strongly suggested an association between *C. pneumoniae* and reactive airway disease in children and adults (3, 5). In our previous study, we noted that children who were on steroid treatment and who were infected with *C. pneumoniae* were infected with remarkably high titers of the organism (3).

Several studies have described the effects of corticosteroids on the growth of bacteria. In 1984, Nash et al. (8) studied the interaction between virulent *Legionella pneumophila* and human alveolar macrophages obtained by bronchoalveolar lavage from healthy adults. Pretreatment of alveolar macrophages with hydrocortisone had no influence on the intracellular multiplication of *L. pneumophila* or on the inhibition of that multiplication by activated alveolar macrophages. Clinically, the severity of illness with *L. pneumophila* appears to be increased in patients who were on prolonged therapy with corticosteroids. North and Izzo (10) studied the kinetics of growth of two virulent and two attenuated strains of *Mycobacterium tuberculosis* in a mouse model. Hydrocortisone treatment enabled attenuated as well as virulent organisms to grow more rapidly in all organs of the mice.

However, information on the effects of steroids on the growth of chlamydial organisms is limited. Previous in vitro studies found a significant increase in the number of inclusions produced from a constant inoculum of *C. trachomatis* in McCoy cells incubated with steroids including hydrocortisone and prednisolone (1). Experiments performed with a mouse model demonstrated reactivation and latent pulmonary infection with *C. trachomatis* in the presence of steroids (12, 13). Recently, Malinverni et al. (7) demonstrated reactivation of *C. pneumoniae* infection of the lung in a mouse model following immunosuppression with cortisone.

The results of the present study demonstrated that the addition of steroids enhanced the growth of two of three strains of *C. pneumoniae* in HEp-2 cells. No dose-response effect was observed. It is interesting that even the presence of fairly low concentrations of hydrocortisone succinate seemed to significantly enhance the growth of *C. pneumoniae* TW-183 and BAL-14 in HEp-2 cells. The highest titers of *C. pneumoniae* TW-183 were seen with 0.25 μg of hydrocortisone succinate per ml, which is considerably lower than the recommended level in serum (1 to 1.5 μg/ml) for the treatment of asthma (2).

TABLE 3. Antimicrobial susceptibility testing of *C. pneumoniae* TW-183, BAL-14, and T2023 against erythromycin, doxycycline, and azithromycin with and without hydrocortisone succinate at three concentrations

Isolate	Drug	MIC (μg/ml)				MCC (μg/ml)			
		Without HS[a]	With HS at concn (μg/ml) of:			Without HS	With HS at concn (μg/ml) of:		
			0.25	1.0	3.0		0.25	1.0	3.0
TW-183	Erythromycin	0.015	0.015	0.015	0.03	0.015	0.03	0.015	0.03
	Doxycycline	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.125
	Azithromycin	0.03	0.03	0.06	0.06	0.03	0.03	0.06	0.06
BAL-14	Erythromycin	0.03	0.06	0.125	0.06	0.06	0.06	0.125	0.125
	Doxycycline	0.06	0.06	0.06	0.125	0.06	0.06	0.06	0.125
	Azithromycin	0.06	0.06	0.06	0.125	0.125	0.125	0.125	0.125
T2023	Erythromycin	0.03	0.06	0.03	0.06	0.125	0.125	0.125	0.25
	Doxycycline	0.06	0.06	0.06	0.125	0.06	0.125	0.06	0.125
	Azithromycin	0.03	0.06	0.06	0.03	0.125	0.125	0.125	0.125

[a] HS, hydrocortisone succinate.

It is unknown where and how hydrocortisone succinate acts on the growth of chlamydia in cells. Bushell and Hobson (1) reported an increased number of inclusions when hydrocortisone succinate was added up to 21 h after infection with *C. trachomatis*. This effect was thought to result from the changes in cellular metabolism rather than increased uptake of infective elementary bodies into cortisol-treated cells. We made a similar observation with the TW-183 strain of *C. pneumoniae*; even when hydrocortisone succinate was added 12 h after inoculation, there was a significant increase in the inclusion counts. This is unlikely to be due only to the rapid uptake of *C. pneumoniae* by the host cell, since *C. pneumoniae* enters the cell within minutes. There is inhibition of lysosome-phagosome fusion. Cortisol has a stabilizing effect on the lysosomal membrane (1, 2) and may act to stabilize autophagosomes and suppress the premature release of infectious particles. Thus, hydrocortisone succinate may have a direct effect on HEp-2 cells and modify host susceptibility to *C. pneumoniae* independent of the immune system.

The addition of various concentrations of hydrocortisone succinate did not affect the activity of erythromycin, azithromycin, or doxycycline against *C. pneumoniae* in vitro. These preliminary findings support our clinical observations and suggest that the use of steroids in the absence of appropriate antibiotic treatment may have a deleterious effect on asthmatic patients with known *C. pneumoniae* infections. Treatment of *C. pneumoniae* infections should not be affected by concurrent treatment with steroids.

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REFERENCES

1. **Bushell, A. C., and D. Hobson.** 1978. Effect of cortisol on the growth of *Chlamydia trachomatis* in McCoy cells. Infect. Immun. **21:**946–953.
2. **Cochrane, G. M.** 1983. Systemic steroids in asthma, p. 103–120. *In* T. J. H. Clark (ed.), Steroids in asthma: a reappraisal in the light of inhalation therapy, 1st ed. ADIS Press, Auckland, New Zealand.
3. **Emre, U., P. M. Roblin, M. Gelling, W. Dumornay, M. Rao, M. R. Hammerschlag, and J. Schachter.** 1994. The association of *Chlamydia pneumoniae* infection and reactive airway disease in children. Arch. Pediatr. Adolesc. Med. **148:**727–732.
4. **Grayston, J. T.** 1992. Infections caused by *Chlamydia pneumoniae* strain TWAR. Clin. Infect. Dis. **15:**757–763.
5. **Hahn, D. L., R. W. Dodge, and R. Golubjatnikov.** 1991. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. JAMA **266:**225–230.
6. **Hammerschlag, M. R., K. Chirgwin, P. M. Roblin, M. Gelling, W. Dumornay, L. Mandel, P. Smith, and J. Schachter.** 1992. Persistent infection with *Chlamydia pneumoniae* following acute respiratory illness. Clin. Infect. Dis. **14:**178–182.
7. **Malinverni, R., C.-C. Kuo, L. A. Campbell, and J. T. Grayston.** 1995. Reactivation of *Chlamydia pneumoniae* lung infection in mice by cortisone. J. Infect. Dis. **172:**593–594.
8. **Nash, T. W., D. M. Libby, and M. A. Horwitz.** 1984. Interaction between the Legionnaires' disease bacterium (*Legionella pneumophila*) and human alveolar macrophages. J. Clin. Invest. **74:**771–782.
9. **National Institutes of Health.** 1991. National asthma education program coordinating committee: guidelines for the diagnosis and management of asthma. U.S. Department of Health and Human Services Publication 91-3042A. U.S. Government Printing Office, Washington, D.C.
10. **North, R. J., and A. A. Izzo.** 1993. Mycobacterial virulence. Virulent strains of *Mycobacterium tuberculosis* have faster in vitro doubling times and are better equipped to resist growth-inhibiting functions of macrophages in the presence and absence of specific immunity. J. Exp. Med. **177:**1723–1733.
11. **Roblin, P. M., W. Dumornay, and M. R. Hammerschlag.** 1992. Use of HEp-2 cells for improved isolation and passage of *Chlamydia pneumoniae*. J. Clin. Microbiol. **30:**1968–1971.
12. **Stephens, R. S., W.-J. Chen, C.-C. Kuo.** 1982. Effects of corticosteroids and cyclophosphamide on a mouse model of *Chlamydia trachomatis* pneumonitis. Infect. Immun. **35:**680–684.
13. **Yang, Y.-S., C.-C. Kuo, and W.-J. Chen.** 1983. Reactivation of *Chlamydia trachomatis* lung infection in mice by cortisone. Infect. Immun. **39:**655–658.