Sulfated Polysaccharides and a Synthetic Sulfated Polymer Are Potent Inhibitors of Chlamydia trachomatis Infectivity In Vitro but Lack Protective Efficacy in an In Vivo Murine Model of Chlamydial Genital Tract Infection

HUA SU AND HARLAN D. CALDWELL*

Laboratory of Intracellular Parasites, Immunology Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratory, Hamilton, Montana 59840

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Heparin, dextran sulfate, pentosan polysulfate, and a sulfated synthetic copolymer of acrylic acid and vinyl alcohol were shown to be potent inhibitors of Chlamydia trachomatis infectivity for cultured human epithelial cells. Despite their potent antichlamydial activity in vitro, neither heparin nor dextran sulfate was effective in inhibiting the infectivity of C. trachomatis in a murine model of chlamydial infection of the female genital tract.

Genital tract infection caused by the obligate intracellular bacterium Chlamydia trachomatis is the most common sexually transmitted disease (STD) in the United States (15, 16). In women, ascending infections of the genital tract can produce serious sequelae that include pelvic inflammatory disease, ectopic pregnancy, and reproductive disability (4, 5, 9, 12). It is estimated that the cost of treating chlamydial infections and their sequelae in the United States alone approaches $2.2 billion annually. Control of chlamydial STDs has focused on four areas of intervention strategies: (i) improved diagnosis and treatment of subclinical infections, (ii) behavioral modification, (iii) vaccination, and (iv) microbicides. Because many chlamydial infections are subclinical, early antibiotic intervention has not been highly effective in controlling chlamydial STDs. Although advances are being made in understanding the immune mechanism(s) that confers protection against chlamydial genital tract infection in animal models, an efficacious vaccine for use in humans does not appear to be forthcoming in the near future. Antichlamydial microbicides have been implicated as one of the most promising control measures, particularly for women, since they could be easily administered intravaginally prior to sexual intercourse.

We (19) and others (22, 23) have shown that the glycosaminoglycans are potent inhibitors of chlamydial infectivity for cultured human cervical epithelial cells. Although currently controversial, we have proposed that chlamydial attachment to host cells is mediated through the specific binding of the chlamydial major outer membrane protein to host cell glycosaminoglycan receptors of the heparan sulfate family (19). Chlamydial attachment to epithelial cells is an initial and critical step in the pathogenesis of infection; therefore, irrespective of the mechanism(s) employed, inhibition of chlamydial adherence to cervical epithelial cells by vaginally administered glycosaminoglycans or structurally similar compounds that exhibit antichlamydial activity would represent a plausible approach for preventing or controlling chlamydial infections in women.

In this study, we have investigated sulfated polysaccharides such as heparin, dextran sulfate (DS), pentosan polysulfate (PPS), and a sulfated synthetic polymer as potential antichlamydial microbicides. These compounds have been reported to have inhibitory effects on other STD pathogens, such as human immunodeficiency virus (1–3, 11, 13, 20), herpes simplex virus (14), and Neisseria gonorrhoeae (8, 21). Our findings show that these compounds are also potent inhibitors of chlamydial infectivity in vitro; however, they are not efficacious as antichlamydial microbicides in vivo models of chlamydial infection of the female genital tract.

Chlamydiae. The C. trachomatis strain UW-31 (serovar D) and the mouse pneumonitis (MoPn) strain were grown in HeLa 229 cells, and elementary bodies (EBs) were purified and inclusion-forming units (IFUs) were determined as previously described (6).

Sulfated polysaccharides and synthetic sulfated polymer. The compounds used were heparin, DS (Mw, 1,000 [DS-1,000]; Mw, 5,000 [DS-5,000]; Mw, 10,000 [DS-10,000]), PPS (Sigma Chemical Co., St. Louis, Mo.), and a copolymer of acrylic acid with vinylalcohol sulfate (ratio, 1:9; 50% sulfonation of hydroxyl groups), referred to hereafter in this work as PAVAS, obtained from E. de Clercq (Rago Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium) through John Swanson (Rocky Mountain Laboratory, Hamilton, Mont.).

In vitro antichlamydial activity. C. trachomatis serovar D and MoPn EBs were diluted in 10 mM sodium phosphate–0.25 M sucrose–5 mM glutamic acid (SPG), pH 7.4, to contain 6 × 10^9 and 5 × 10^9 IFUs, respectively. Serial 10-fold dilutions (200 to 0.02 μg/ml) of each compound were prepared in SPG, and equal volumes of the diluted compounds were mixed with the chlamydial suspensions. A 200-μl volume of this mixture was inoculated onto washed HeLa 229 monolayers and incubated at 37°C for 2 h. The monolayers were washed, refed with medium, and incubated for an additional 30 h at 37°C. The monolayers were then washed and fixed with methanol, and chlamydial inclusions were stained and visualized by indirect immunofluorescence using a genus-specific antilipopolysaccharide monoclonal antibody (EVI-H1). The inhibitory activity of compounds is expressed as percent reduction of IFUs and was calculated as previously described (17).

The dose response inhibition of infectivity and the 50% inhibitory concentration (IC50) of each compound for serovar D and MoPn EBs assayed on HeLa 229 cells are shown in Fig. 1. Heparin, DS-1000, DS-5000, DS-10,000, PPS, and PAVAS...
all exhibited a dose-dependent inhibition of infectivity for both C. trachomatis strains. Maximal inhibition of infectivity (95% or greater reduction in IFUs) for both chlamydial strains was obtained at compound concentrations ranging from 1 to 10 µg/ml. The IC₅₀ₐₗₙₐ of all compounds for chlamydial infectivity of HeLa 229 cells were very similar, ranging from 0.02 to 0.12 µg/ml. Inhibition of infectivity was not related to the Mₘₐₚₚₜ of polysulfated compounds, as DS-1,000, DS-5,000, and DS-10,000 were equally effective in their inhibitory properties, nor were there differences in the inhibitory activities among sulfated polysaccharides and PAVAS (a sulfated synthetic polymer). These findings demonstrate that sulfated polysaccharides and PAVAS are potent inhibitors of chlamydial infectivity in vitro and therefore warrant further experimentation to evaluate their ability to function as inhibitors of chlamydial infectivity in vivo.

**In vivo antichlamydial activity.** For in vivo studies, 6- to 8-week-old female C57BL/10 mice (Jackson Laboratory, Bar Harbor, Maine) were used. Mice were given food and water ad libitum and were maintained under Association for Assessment and Accreditation of Laboratory Animal Care-accredited housing conditions. The chlamydial challenge strain used for these studies was MoPn. It was selected because of its well-documented infectivity properties in the murine model. Heparin and DS-10,000 were tested as inhibitors in in vivo assays of chlamydial infectivity. Two experimental approaches were investigated to assess the potential inhibitory activity of these compounds in vivo. The first was to preincubate chlamydiae with each compound in vitro and then challenge mice intra-vaginally, and the second was to administer the compounds alone into the vaginal vaults of mice, followed immediately by an infectious chlamydial challenge. For the first experiment, 50 µl of MoPn EBs (6 x 10⁷ IFUs/ml) was mixed with 50 µl of SPG containing either heparin or DS-10,000 (2 or 100 mg/ml). A 5-µl volume of the mixture (1,500 IFUs equals 100 50% infectious doses [ID₅₀ₐₗₐ]) was then inoculated into the vaginal vaults of 5 mice. In the second experimental group, 20 µl of heparin or DS-10,000 (100 mg/ml in SPG) was inoculated into the vaginal vaults of five mice; this was immediately followed by a 5-µl (100 ID₅₀ₐₗₐ) challenge of MoPn EBs. Control mice for each group either were inoculated intravaginally with 5 µl (100 ID₅₀ₐₗₐ) of untreated MoPn EBs or received 20 µl of SPG intravaginally prior to chlamydial challenge. Mice received subcutaneous injections of 2.5 mg of medroxyprogesterone acetate (Depo-Provera; Upjohn, Kalamazoo, Mich.) in 100 µl of saline 10 and 3 days prior to challenge to synchronize estrus. The kinetics of infection were monitored by swabbing the vaginal vault with Calgiswabs (Spectrum Medical Industries, Los Angeles, Calif.) at selected intervals after challenge. Recoverable organisms were titrated on HeLa 229 cell monolayers as described previously (18).

The percentage of mice infected and the recovery of chlamydiae from cervico-vaginal swabs of mice in each experimental group are shown in Fig. 2. Figure 2A and B show the effect of preincubation of chlamydiae with either heparin or DS-10,000 (2 mg/ml) prior to intravaginal challenge of mice. There was no effect of either compound on the percentage of mice infected, cervico-vaginal shedding of chlamydiae, or duration of infection. Increasing the concentration of either compound (100 mg/ml) was also without effect on chlamydial infectivity (data not shown). Figure 2C shows the effect of instillation of each compound into the vaginal vault prior to infectious chlamydial challenge. Similarly, the presence of either compound in the vaginal vault prior to challenge had no demonstrable effect on the percentage of mice infected or the kinetics of infection. Thus, although both heparin and DS-10,000 exhibited potent antichlamydial inhibitory activity in vitro neither compound was effective in preventing chlamydial infection in vivo. This lack of efficacy was surprising since the concentrations of heparin and DS-10,000 were 100- to 5,000-fold greater than that required to achieve maximum inhibition of chlamydial infectivity in vitro (Fig. 1). There are several possible explanations for these disparate results. The first possibility is that inhibition of infectivity was incomplete, and the small number of infectious organisms that were not inhibited by the compounds was sufficient to establish a productive infection in...
vivo. This may in fact be the case, since as few as 15 IFUs of the MoPn EBs are sufficient to infect mice intravaginally (10). A second possibility is that differences exist in vitro and in vivo either the chlamydial ligand or their cognate host receptor(s) that functions in chlamydial adherence to epithelial cells. The latter possibility is supported in part by the recent work of Chen and Stephens (7), showing that chlamydial adherence to epithelial cells occurs through both glycosaminoglycan-dependent and -independent mechanisms. Thus, chlamydial adherence in vivo might be mediated through a glycosaminoglycan-independent mechanism(s) which would not be expected to be inhibited by heparin or DS-10,000. It is also possible that delivery methods that would provide a sustained concentration of inhibitory compounds at the genital tract mucosa would yield more favorable results than those reported here. Regardless, our findings emphasize the importance of testing candidate anti-chlamydial microbicides in vivo models of chlamydial infection to more accurately assess their utility for the potential control of chlamydial STDs in humans.

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REFERENCES


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