



OPEN ACCESS

# Polyphenylene carboxymethylene (PPCM), the active component of the topical contraceptive Yaso-GEL, exhibits potent antimicrobial activity against *Neisseria gonorrhoeae* in preclinical studies

Michelle Pilligua-Lucas,<sup>1,2</sup> Rok Tkavc,<sup>1,2</sup> Sarah K Bash,<sup>1,2</sup> Barbara B North,<sup>3</sup> Mary B Weitzel,<sup>3</sup> Ann E Jerse <sup>2</sup>

► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/sextrans-2022-055596>).

<sup>1</sup>Henry M Jackson Foundation for the Advancement of Military Medicine, Bethesda, Maryland, USA

<sup>2</sup>Department of Microbiology and Immunology, Uniformed Services University, Bethesda, Maryland, USA

<sup>3</sup>Yaso Therapeutics, Inc, Frisco, Texas, USA

## Correspondence to

Dr Ann E Jerse, Department of Microbiology and Immunology, Uniformed Services University, Bethesda, MD 20814, USA; [ann.jerse1@usuhs.edu](mailto:ann.jerse1@usuhs.edu)

Received 8 August 2022

Accepted 7 January 2023

Published Online First

8 May 2023



© Author(s) (or their employer(s)) 2023. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

**To cite:** Pilligua-Lucas M, Tkavc R, Bash SK, et al. *Sex Transm Infect* 2023;**99**:409–415.

## ABSTRACT

**Introduction** Polyphenylene carboxymethylene (PPCM) is a condensation polymer that has both contraceptive and antimicrobial activity against several sexually transmitted viruses including HIV, herpes simplex virus, Ebola virus and SARS-CoV-2 in preclinical studies. PPCM, both as an active pharmaceutical ingredient (API) and in a vaginal gel formulation (Yaso-GEL), has an excellent safety profile. Here, we evaluated the efficacy of PPCM against *Neisseria gonorrhoeae* in vitro and in a gonorrhoea mouse model.

**Methods** The minimal inhibitory concentration (MIC) of PPCM was determined against 11 *N. gonorrhoeae* strains by agar dilution and a microtitre plate-based method. In vivo efficacy was tested in a murine model of *N. gonorrhoeae* genital tract infection by applying Yaso-GEL, PPCM incorporated in 2.7% hydroxyethylcellulose (HEC), or the HEC vehicle vaginally prior to challenge with *N. gonorrhoeae*. Vaginal swabs were quantitatively cultured over 5 days to assess efficacy.

**Results** PPCM MIC against *N. gonorrhoeae* ranged between 5–100 µg/mL (agar dilution) and 50–200 µg/mL (microtitre plate method). PPCM/HEC gel applied vaginally prior to bacterial challenge resulted in a concentration-dependent inhibition of infection. Yaso-GEL containing 4% PPCM prevented infection in 100% of mice. Incubation of *N. gonorrhoeae* with PPCM increased membrane permeability, suggesting PPCM directly compromises *N. gonorrhoeae* viability, which may be a mechanism by which PPCM inhibits *N. gonorrhoeae* infection.

**Conclusions** Yaso-GEL containing the API PPCM showed significant activity against *N. gonorrhoeae* in vitro and in vivo in a female mouse model. These data support further development of Yaso-GEL as an inexpensive, non-hormonal and non-systemic product with both contraceptive and antimicrobial activity against gonorrhea and other common sexually transmitted infections (STIs). Such multipurpose prevention technology products are needed by women in all economic, social and cultural circumstances to prevent unintended pregnancy and STIs.

## BACKGROUND

Over 82 million *Neisseria gonorrhoeae* infections are estimated to occur globally each year.<sup>1</sup> The primary site of gonococcal infection is the urethra

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Control of gonorrhea is seriously threatened by the emergence of multidrug-resistant *Neisseria gonorrhoeae*. Polyphenylene carboxymethylene (PPCM), the active component of Yaso-GEL, is both a contraceptive and antimicrobial compound with activity against other sexually transmitted pathogens.

## WHAT THIS STUDY ADDS

⇒ PPCM has potent activity against multiple strains of *N. gonorrhoeae* in vitro and when used prophylactically in a mouse model.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study supports further development of PPCM as a safe, inexpensive and non-hormonal multipurpose technology that offers women protection from several sexually transmitted infections including gonorrhea.

in males and the cervix and/or urethra in females; nasopharyngeal and rectal infections are also common. Women and neonates born to infected mothers suffer the most serious morbidity and mortality associated with gonorrhea. Both acute and silent *N. gonorrhoeae* cervical infections can ascend to cause pelvic inflammatory disease, which is associated with ectopic pregnancy, infertility and chronic pelvic pain. Maternal *N. gonorrhoeae* is associated with premature rupture of membranes, low birthweight babies and a failure to thrive and transmission during delivery can result in acute conjunctivitis.<sup>2</sup> Individuals with gonorrhea are more susceptible to HIV and may also transmit HIV more readily due to higher levels of HIV transcripts in their genital fluids.<sup>3</sup>

Control of gonorrhea is limited to safe-sex counselling and the identification and treatment of infected individuals and their sexual contacts. Treatment, however, is seriously threatened by the emergence of antibiotic-resistant *N. gonorrhoeae* strains, particularly in low-to-middle-income countries where the diagnosis is often empirical and antibiotic use is less regulated.<sup>4</sup> Several candidate gonorrhea vaccines are under development; however, no

vaccine is yet licensed for gonorrhea. While a gonorrhea vaccine is a desirable public health tool to prevent disease and curb the evolution of antibiotic resistance, multipronged interventions will always be needed to protect individuals in areas where vaccines are not available and to provide an alternative for those with vaccine hesitancy.

One approach for preventing sexually transmitted infections (STIs) in women and transgender females is the use of vaginally applied microbicides. Advantages of this strategy include low production costs and giving the female partner control over their use. Multipurpose prevention technology products (MPTs) that have both microbicidal and contraceptive activity are also highly desirable.<sup>5</sup> The addition of contraceptive activity to an STI prevention product is expected to increase its acceptability and actual use. Polyphenylene carboxymethylene (PPCM), the active pharmacological ingredient of Yaso-GEL, is a condensation polymer of mandelic acid and an inexpensive topical contraceptive agent.<sup>6,7</sup> PPCM also has microbicidal activity against sexually transmitted viruses, including HIV,<sup>8,9</sup> herpes simplex virus (HSV),<sup>8–10</sup> Ebola virus<sup>11</sup> and SARS-CoV-2.<sup>12</sup> Importantly, PPCM has a good safety profile in preclinical toxicity assays and is not cytotoxic or damaging to epithelial cells.<sup>7,8</sup> Here we evaluated the in vitro and in vivo efficacy of PPCM as a topical microbicide against *N. gonorrhoeae* to more fully define the protective potential of Yaso-GEL against STI pathogens.

## METHODS

### Materials

PPCM is a condensation polymer with a molecular weight of 3900 g/mol and a polydispersity index of 1.4 and is licensed to Yaso Therapeutics. PPCM is highly soluble in water and stable.<sup>6</sup> PPCM was synthesised by Wilmington PharmaTech (Newark, Delaware, USA) under cGMP for Yaso Therapeutics. PPCM agar solutions were produced by the addition of PPCM to supplemented GC agar (36 g GC medium base, 5 g Bacto agar per liter of dH<sub>2</sub>O) containing Kellogg's Supplement I<sup>13</sup> and 12 µM Fe(NO<sub>3</sub>)<sub>3</sub>) as described.<sup>14</sup> Yaso-GEL is a 4% (40 mg/mL) PPCM aqueous gel, produced by Dow Development Labs (Petaluma, California, USA) for Yaso Therapeutics. PPCM HEC (hydroxyethyl cellulose) gel of 2 mg/mL was prepared by combining 20 mg PPCM and 27 mg HEC in 10-mL endotoxin-free phosphate-buffered saline (PBS) and stirring for 10–15 min until a 2.7% gel was formed. Gynol-II (DPT Laboratories) is a commercially available spermicidal gel that contains 2% nonoxonyl-9 (N-9).

### Strains and growth conditions

*N. gonorrhoeae* strains used in this study were two laboratory strains (FA1090, MS11), two ceftriaxone-resistant strains (H041, F89), four well-characterised WHO reference strains and three *N. gonorrhoeae* isolates isolated from the USA between 2014 and 2019 (table 1). *N. gonorrhoeae* was cultured in 7% CO<sub>2</sub> at 37°C on supplemented GC agar. GC agar with antibiotic selection (vancomycin, colistin, nystatin, trimethoprim and streptomycin (GC-VCNTS agar)) and heart infusion agar were used to isolate *N. gonorrhoeae* and murine commensal microbiota from murine vaginal mucus as described.<sup>14</sup> All bacterial culture media were from Difco (Becton Dickinson).

### Minimal inhibitory concentration against *N. gonorrhoeae*

For agar dilution assays, twofold decreasing concentrations of PPCM were added to cooled (55°C) GC agar and 6 mL of each concentration were poured into a separate well of a 6-well tissue

**Table 1** Minimal inhibitory concentration (MIC) of PPCM against the *Neisseria gonorrhoeae* strains used in this study as determined by agar dilution and microtitre plate assays

| Strain        | Susceptibility profile  | MIC (µg/mL)   |                  |
|---------------|---|---------------|------------------|
|               |   | Agar dilution | Microtitre plate |
| FA1090*       | STM <sup>R</sup>  | 25            | 100              |
| MS11*         | STM <sup>R</sup>  | 100           | 200              |
| H041 (WHO X)† | PEN-G <sup>R</sup> , TET <sup>R</sup> , AXI <sup>I</sup> , CIP <sup>HLR</sup> , CFX <sup>HLR</sup> , CRO <sup>HLR</sup> | 25            | 100              |
| F89†          | Pen-G <sup>R</sup> , TET <sup>R</sup> , CFX <sup>HLR</sup> , CRO <sup>HLR</sup> , CIP <sup>HLR</sup>                    | ≤5            | 50               |
| WHO F†        | susceptible   | ≤5            | 50               |
| WHO G†        | PEN-G <sup>I</sup> , TET <sup>R</sup> , CIP <sup>LLR</sup>  | ≤5            | 50               |
| WHO K†        | PEN-G <sup>R</sup> , TET <sup>R</sup> , CIP <sup>HLR</sup> , CFX <sup>LLR</sup>   | 100           | 100              |
| WHO L†        | PEN-G <sup>R</sup> , TET <sup>R</sup> , CIP <sup>HLR</sup> , CRO <sup>LLR</sup>   | 100           | 200              |
| CONUS-9542‡   | TET <sup>R</sup> , CIP <sup>R</sup>   | 100           | Not determined   |
| CONUS-3668‡   | PEN <sup>R</sup> , CIP <sup>R</sup>   | ≤5            | Not determined   |
| CONUS-6364‡   | PEN <sup>R</sup> , CIP <sup>R</sup>   | 25            | Not determined   |

\*Commonly used laboratory *N. gonorrhoeae* strains.<sup>14</sup>

†Multidrug-resistant, ceftriaxone-resistant *N. gonorrhoeae* strains.<sup>27,28</sup>

‡US isolates; from the USU GC Resistance Repository and Reference Laboratory isolated in 2017 (CONUS-9542), 2019 (CONUS-3668) and 2014 (CONUS-6364). AZI, azithromycin; CFX, cefixime; CIP, ciprofloxacin; CRO, ceftriaxone; HLR, high-level resistant; I, intermediate susceptible; LLR, low-level resistant; N/A, not available; PEN G, penicillin G; PPCM, polyphenylene carboxymethylene; R, resistant; STM, streptomycin; TET, tetracycline.

culture plate. Control wells consisted of media without antibiotics. Well-isolated colonies of the *N. gonorrhoeae* strain tested were harvested from GC agar plates after 20- to 22-hour incubation and suspended in GC broth (GCB) to a concentration of 10<sup>7</sup> colony-forming units (CFUs) per millilitre. Ten microlitres of each suspension (ca. 10<sup>5</sup> CFU) were spotted onto the agar in each well with up to five spots per well. Plates were scored for growth after 24-hour incubation. Each strain was tested in triplicate within each of the two independent experiments.

For the microtitre plate-based assay, *N. gonorrhoeae* colonies were harvested from GC agar plates after 20-hour incubation and suspended in 10 mL of supplemented GCB. Bacterial suspensions were passed through a 1.2-µm filter to remove bacterial aggregates, and the optical density at 600 nm (OD<sub>600</sub>) was adjusted to 0.08. Filtered suspensions were then incubated in T25 tissue culture flasks at 150 rpm for 3 hours at 35.5°C, after which the OD<sub>600</sub> of the cultures was adjusted again to 0.08 (~10<sup>7</sup> CFUs/mL). The suspension was then diluted 1:50 in GCB, and 100 µL (~10<sup>5</sup> CFU) was added to wells of a microtitre plate containing 100 µL of serial dilutions of PPCM in supplemented GCB or supplemented GCB without PPCM. The microtitre plates were incubated at 24 hours at 35.5°C in 7% CO<sub>2</sub>, after which 5 µL from each well were inoculated onto GC agar plates. The plates were scored for growth after 24-hour incubation. Two independent iterations of the assay were conducted with three technical replicates tested in each assay. The MIC of Yaso-GEL (4% PPCM) against *N. gonorrhoeae* was tested in microtitre plates similarly, using a positive displacement pipette to dilute the gel. For both the agar dilution and microtitre plate-based assays, the MIC was the lowest concentration of PPCM from which no *N. gonorrhoeae* strains were isolated, and each assay was performed

twice on different days, with each strain tested in triplicate in each experiment.

### Cell permeability assay

The effect of PPCM on cellular membranes was assessed using the SYTO9/propidium iodide (PI) counterstain assay as follows. *N. gonorrhoeae* strain MS11 was harvested from GC agar plates after 19-hour incubation and suspended in GCB to an  $OD_{600}=0.08$ . Aliquots of the suspension were incubated with PPCM (100 µg/mL) or no PPCM for 6 hours. At hourly time points, aliquots from each culture were quantitatively cultured for *N. gonorrhoeae* or incubated in the dark with PI and SYTO9 (1:1 mix from LIVE/DEAD kit) using 100 µL of bacterial suspension with 0.2 µL of PI and SYTO9. After 30-min incubation at room temperature, the stained suspensions were examined under fluorescent microscopy at 40× using the green and blue filters. Photos were taken in three to five different fields of triplicate samples from each preparation at each time point, and stained cells were counted using Image J. The per cent permeability was calculated as the [(no of compromised cells divided by the total number) × 100], with the green signal showing total membranes and the red signal showing compromised membranes. Differences were analysed by ordinary two-way analysis of variance (ANOVA) using computations that assume that all rows are sampled from populations with the same scatter (SD). Differences in the number of CFU recovered over time were measured by repeated measures ANOVA (GraphPad Software, La Jolla, California, USA). Differences  $\leq 0.05$  were considered significant. The assay was performed three times and with three technical replicates in each assay.

### In vivo efficacy testing

The method used to test PPCM efficacy against *N. gonorrhoeae* is described in the online supplemental material file, and a schematic of the protocol is shown in online supplemental figure S1. Briefly, female BALB/c mice were randomised and treated with Premarin and antibiotics to promote the susceptibility to *N. gonorrhoeae*. Two days after Premarin treatment was initiated, mice were anaesthetised and inoculated vaginally with 30 µL of different concentrations of PPCM in 2.7% HEC, 2.7% HEC alone (vehicle control), Gynol-II, Yaso-GEL or a placebo gel using a positive displacement pipette. Thirty seconds later, mice were challenged vaginally with 10 µL of a PBS suspension containing  $10^5$  CFU of pilated *N. gonorrhoeae* strain MS11. Vaginal swabs were quantitatively cultured for *N. gonorrhoeae* on days 1, 2 and 5 postinoculation. The percentage of culture-positive mice at each time point was plotted as a Kaplan-Meier curve, and the results for each group were compared by the log-rank test with Bonferroni correction. The average numbers of CFU isolated per vaginal swab suspension from each experimental group were compared over time by repeated measures ANOVA. A total of 67 mice were used in this study, with experimental groups consisting of 5–7 mice/group. Sample size was determined based on our previous study that showed 6–8 mice/group was adequate to detect a significant difference ( $p < 0.05$ ) in percentage of mice colonised for the moderately active microbicide methylcellulose.<sup>15</sup>

### Animal studies declaration

Animal experiments were conducted at the Uniformed Services University, a facility fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, under

protocol MIC-20-013, which was approved by the University's Institutional Animal Care and Use Committee.

## RESULTS

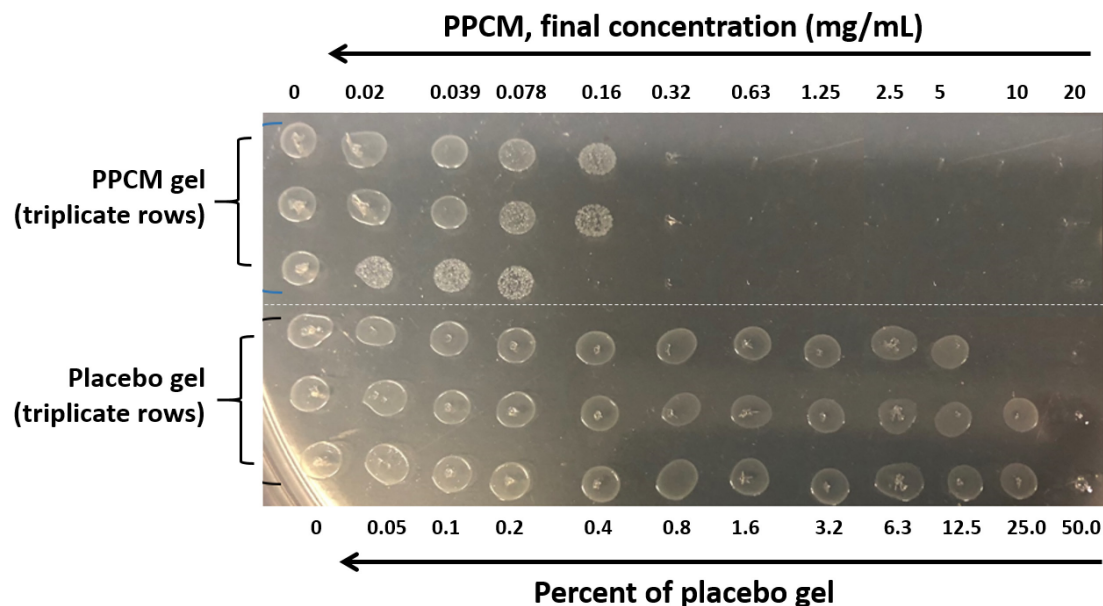
### PPCM in solution and formulated gel has potent activity against *N. gonorrhoeae* in vitro and in vivo

MIC of PPCM was determined against 11 *N. gonorrhoeae* strains that differ in antibiotic susceptibility (table 1). Using the agar dilution method, the MIC of PPCM ranged from  $<5$  to 100 µg/mL. MICs were also determined using a microtitre plate method in which logarithmic-phase bacteria were incubated with decreasing concentrations of PPCM. PPCM also showed activity in this assay, with MIC values 2- to 10-fold higher (50–200 µg/mL) compared with the agar dilution method. We also determined that the MIC of Yaso-GEL ranged between 0.31 and 0.63 mg/mL against strain MS11 using the microtitre plate-based method (figure 1). We conclude that PPCM in aqueous solution and in Yaso-GEL are highly inhibitory against *N. gonorrhoeae*, with no differences in PPCM susceptibility observed among a diverse set of *N. gonorrhoeae* strains.

Based on these results, we next tested the in vivo efficacy of 2 mg/mL against *N. gonorrhoeae* strain MS11, which is  $10\times$  the in vitro MIC against this strain. For these experiments, mice were vaginally inoculated with PPCM mixed with 2.7% HEC to create a viscous formulation that would be retained longer in the vagina or HEC alone prior to the *N. gonorrhoeae* challenge. We tested the commercial spermicide Gynol-II in parallel, which contains 2% of N-9, which is a detergent that kills *N. gonorrhoeae*, but unlike Yaso-GEL, is highly toxic to host cells.<sup>16</sup> No *N. gonorrhoeae* was recovered from any of the seven mice in the 2 mg/mL PPCM-HEC gel-treated group 24 hours after challenge or at any time point through 5 days; in comparison, 100% (6/6) of mice in the vehicle control group were culture-positive for 2 consecutive days following bacterial challenge ( $p=0.008$ ) and 50% (3/7) were culture-positive on day 5 (figure 2A). The difference in the number of recoverable CFUs/100 mL vaginal swab suspension over time was also significantly lower in PPCM-treated mice versus HEC-treated mice ( $p \leq 0.0001$ ) (figure 2B). Fifty per cent (3/6) of mice in the Gynol-II group were culture-positive on days 1, 2 and 5 postchallenge, which was significantly higher than the PPCM-treated group ( $p=0.04$ ) as was the average number of CFUs/mL recovered over time ( $p=0.03$ ) (figure 2A,B, purple lines).

To determine the lowest effective dose of PPCM in vivo, we pretreated mice with PPCM-HEC containing 0.25–2.0 mg/mL PPCM. Pretreatment with 1.0 and 0.5 mg/mL PPCM-HEC was significantly more effective than the HEC vehicle alone as shown by the percentage of culture-positive mice over time ( $p=0.03$  and  $p=0.05$ , respectively) (figure 2C, gold and light blue lines) and the average number of CFUs/mL recovered ( $p=0.07$  and  $p=0.004$ , respectively) (figure 2D). Mice treated with the 0.25 mg/mL dose showed an initial drop in the percentage of culture-positive mice and CFUs/mL recovered on day 1 but were similar to the HEC vehicle control for both parameters on subsequent time points (green line, figure 2C and D). The 2 mg/mL dose did not perform as well as in the first experiment, with 40% (2/5) of the mice colonised over 5 days ( $p=0.19$  vs vehicle control) compared with 0% (0/7) mice in the first study (figure 1C, red line) and the difference in the recoverable bioburden approaching, but not achieving statistical significance ( $p=0.07$ ). We next tested the in vivo efficacy of Yaso-GEL. The results showed Yaso-GEL to be highly effective compared with the placebo gel in reducing both the percentage of culture-positive mice over time ( $p=0.002$ )





**Figure 1** Yaso-GEL exhibits potent activity against *Neisseria gonorrhoeae* in vitro. The minimum inhibitory concentration of Yaso-GEL, which contains PPCM at a concentration of 40 mg/mL, was determined by incubating  $10^5$  CFUs of *N. gonorrhoeae* strain FA1090 with twofold dilutions of the formulated gel in a microtitre plate (moving right to left on the plate as shown above) for 24 hours. Five-microlitre aliquots were then spotted onto GC agar, incubated for 24 hours, and scored for growth. The lowest dilutions of Yaso-GEL completely inhibited *N. gonorrhoeae* ranged from 1:16 to 1:32, which corresponds to PPCM concentrations of 0.16–0.32 mg/mL. The placebo gel had no effect at any dilution tested. CFU, colony-forming units; PPCM, polyphenylene carboxymethylene.

(figure 2E) and colonisation load ( $p=0.0005$ ) (figure 2F), with no culture-positive mice detected at any time point (0/7 mice). Gynol-II also showed activity, with 28% (2/7) of the mice in this group culture-positive on days 1 and 2 postbacterial inoculation ( $p=0.041$  compared to HEC). We conclude that PPCM at concentrations  $>0.25$  mg/mL inhibits *N. gonorrhoeae* in vivo and that vaginal application of Yaso-GEL is highly effective in preventing experimental *N. gonorrhoeae* genital tract infection in mice.

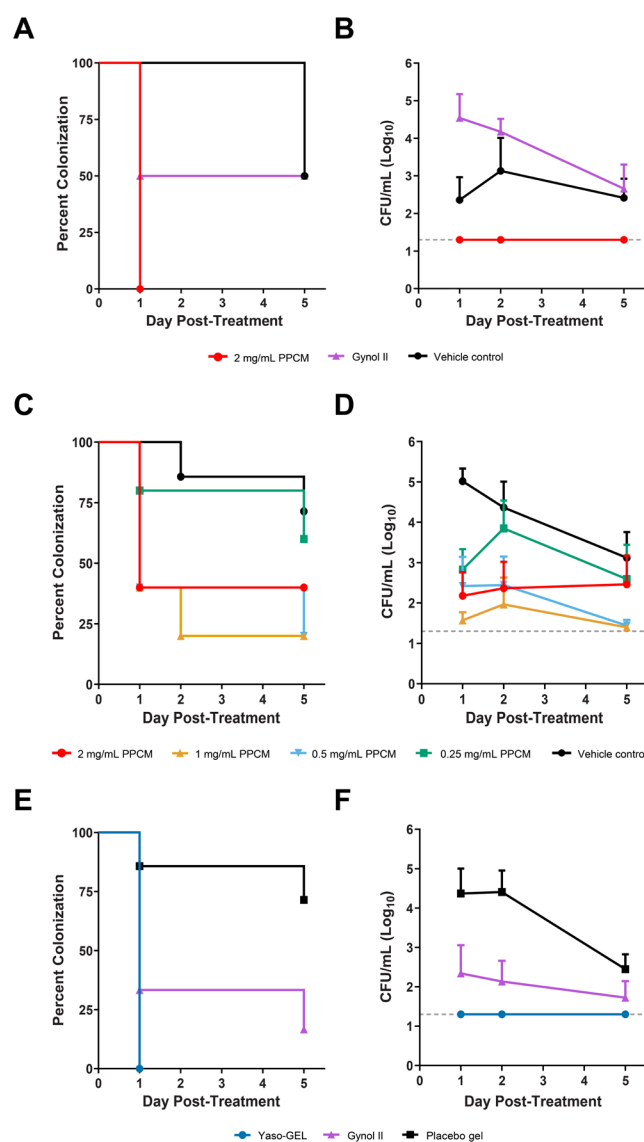
#### PPCM increases bacterial cellular permeability

To examine the mechanism of action of PPCM against *N. gonorrhoeae*, we tested two hypotheses. First, *N. gonorrhoeae* is pH-sensitive, and acidification of the vagina is an effective defence against *N. gonorrhoeae* infection.<sup>15</sup> We therefore compared the pH of PPCM prepared in GCB at concentrations of 1.56  $\mu$ g/mL to 1.6 mg/mL, which are similar to the concentrations tested in the microtiter plate MIC assay and in vivo studies. The pH values ranged from 7.38 to 7.55, with no correlation between pH and PPCM concentration, ruling out pH reduction as a possible mechanism. We next hypothesised that PPCM might make the gonococcal cellular membranes more permeable, leading to a loss in viability. To test this hypothesis, we incubated *N. gonorrhoeae* with 100  $\mu$ g/mL of PPCM and removed aliquots at hourly time points for quantitative culture and staining using the SYTO9/PI counterstain assay. No difference in the per cent permeability of the bacterial membranes was observed after 2 and 3 hours of incubation; however, significantly increased permeability was detected at 1 hour ( $p=0.01$ ) and at 4 and 5 hours of incubation compared with the untreated control ( $p<0.001$ ) (figure 3A). A significant reduction in the average number of CFU recovered from samples incubated with PPCM versus no PPCM occurred in parallel, with a  $>1$  log reduction occurring between 4 and 6 hours (figure 3B). We conclude that PPCM is directly bactericidal to *N. gonorrhoeae*.

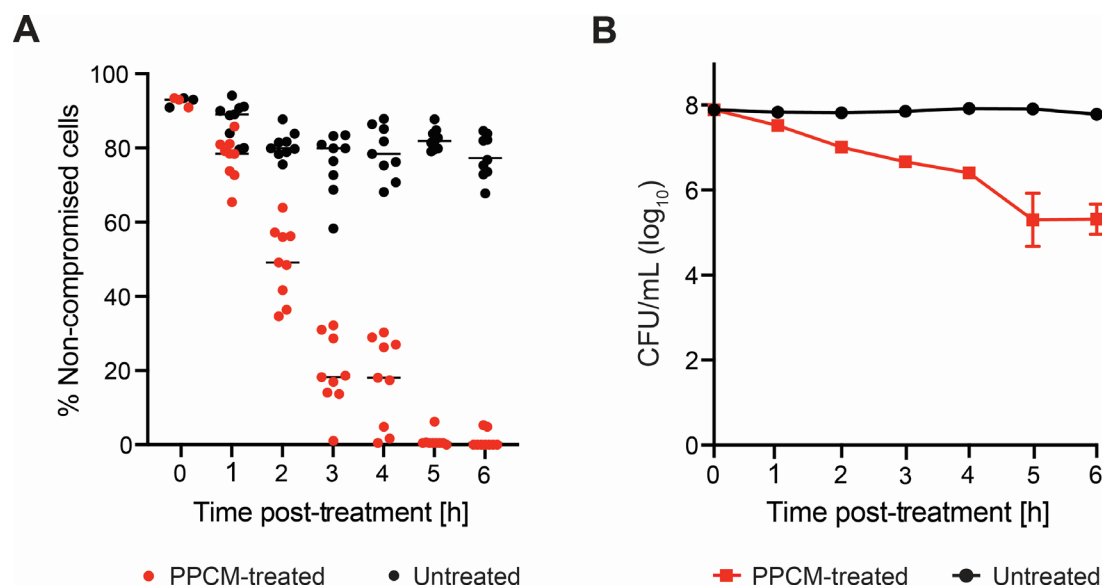
#### DISCUSSION

Protection from STIs and pregnancy protection are both critical aspects of women's health. Currently there are no options that provide dual protection that is under the control of the female partner. In the last decade, the need for dual protection that is woman-controlled has become widely recognised. This unmet need has evolved into an active pipeline for the development of MPTs, defined as an innovative class of products that deliver varied combinations of HIV prevention, other STI prevention and contraception.<sup>5</sup> Currently available MPT methods are limited. Both male and female condoms function as MPTs, but their use has been limited by dissatisfaction/discomfort, cost and availability.<sup>17 18</sup> Early in vitro and limited clinical studies suggested that N-9 might offer some protection against gonorrhea as well as contraception.<sup>19</sup> Unfortunately, the cytotoxic effects of N-9 can actually damage host genital epithelial cells and may even increase the risk of HIV infection.<sup>20</sup> *N. gonorrhoeae* is very sensitive to N-9 in vitro, which is not surprising since it is a surfactant that is cytotoxic and damages cell membranes. In our study, an N-9-containing spermicide was used as a positive control but was not as effective as PPCM in the mouse model.

Many newer vaginal MPT products that are currently in the development pipeline focus on hormonal contraceptives combined with HIV prevention, with relatively little attention to other important STIs.<sup>21</sup> While the extreme morbidity and mortality linked with HIV are widely feared, other STI pathogens, particularly HPV, HSV, *Trichomonas vaginalis*, *Chlamydia trachomatis*, and *N. gonorrhoeae* pose a greater infection risk to many women and their partners.<sup>22</sup> Gonorrhea is particularly problematic not only because of significant global incidence and serious health sequelae, but worsening antibiotic resistance has severely limited treatment options.<sup>23</sup> Only one vaginal product, a recently marketed vaginal contraceptive, is in clinical trials for *N. gonorrhoeae* and *C. trachomatis* prevention.<sup>24</sup>



**Figure 2** PPCM and Yaso-GEL are effective in preventing experimental *Neisseria gonorrhoeae* genital tract infection. The in vivo efficacy of PPCM formulated in 2.7% HEC or Yaso-GEL in preventing *N. gonorrhoeae* infection was tested by applying the test compounds vaginally followed by the challenge with *N. gonorrhoeae* strain MS11. Panels on the left (A, C and E) show the percentage of culture-positive mice on days 1, 2 and 5 postchallenge, and panels on the right (B, D, F) show the average number of *N. gonorrhoeae* CFU recovered from each group for each of three different experiments. In the first experiment, 2% PPCM was significantly effective versus the HEC vehicle when comparing: (A) the percentage of culture-positive mice at each time point ( $p=0.008$ ) and (B) the number of CFUs recovered per millilitre of vaginal swab suspension over time (2 mg/mL PPCM,  $p<0.0001$ ) ( $n=6-7$  mice/group). PPCM was also significantly more effective against *N. gonorrhoeae* than Gynol II, which contains the spermicidal detergent N-9, for per cent colonised ( $p=0.04$ ) and CFUs recovered per millilitre ( $p=0.03$ ). No significant difference in the percentage of mice colonised was detected in the Gynol II-treated and the HEC control groups ( $p=0.64$ ); the difference in CFUs recovered per millilitre was at a level of  $p=0.56$ . (C) Comparison of the percentage of infected mice given decreasing doses of PPCM ( $n=5$  mice/test group, 7 mice in the HEC group) showed that the 1.0 and 0.5 mg/mL doses were more effective than HEC alone ( $p=0.03$  and  $p=0.05$ , respectively). The 2 mg/mL treatment and the 0.25 mg/mL treatment showed no significant difference compared with the vehicle control ( $p=0.07$  and  $p=0.19$ , respectively). (D) The average number of CFUs recovered per millilitre overtime was significantly lower in the 1 mg/mL and 0.5 mg/mL treatment groups compared with HEC alone ( $p=0.007$  and  $p=0.03$ , respectively), but not in the 2 mg/mL dose and 0.25 mg/mL dose ( $p=0.07$  and  $p=0.19$ , respectively). In a third experiment, Yaso-GEL was tested similarly; results showed the gel to be highly effective compared with the placebo gel in reducing (E) the percentage of culture-positive mice over time ( $p=0.002$ ) and (F) the colonisation load ( $p=0.0005$ ) ( $n=7$  mice/group). While Yaso-GEL appeared more effective than Gynol-II, the differences were not statistically different for the percentage of mice colonised over time ( $p=0.11$ ) or log<sub>10</sub> CFU recovered ( $p=0.15$ ). In all panels, bars represent SE of the mean. CFU, colony-forming units; HEC, hydroxyethyl cellulose; N-9, nonoxynol-9; PPCM, polyphenylene carboxymethylene.



**Figure 3** PPCM increases the permeability of cellular membranes. The effect of PPCM on gonococcal cellular membrane integrity and viability was assessed over a 6-hour incubation period. Bacterial suspensions were exposed to PPCM or not exposed, and aliquots were taken at hourly time points and stained with SYTO9/propidium iodide (PI) (panel A) or quantitatively cultured (panel B). The per cent permeability was calculated using the formula  $\frac{\text{No of Compromised}}{\text{No of Total}} \times 100$ , with the green signal showing total membranes and the red signal showing compromised membranes. (A) The percentage of non-compromised cells in samples with PPCM significantly decreased over time compared with samples without PPCM ( $p < 0.0001$ ; ordinary two-way ANOVA), with the most dramatic decrease seen between 3 and 6 hours; (B) a significant reduction in the average number of CFUs recovered from samples incubated with PPCM versus no PPCM occurred over time ( $p = 0.001$ ; repeated measures ANOVA), with a  $>1$  log reduction occurring between 4 and 6 hours. The results are shown for three assays performed in triplicate. ANOVA, analysis of variance; CFUs, colony-forming units; PPCM, polyphenylene carboxymethylene.

In this study, we evaluated the ability of PPCM to prevent infection by *N. gonorrhoeae*. PPCM (aka SAMMA) is a unique (non-sulfated/non-sulfonated) polyanion developed by the Topical Prevention of Conception and Disease (TOPCAD) at Rush University Medical Center and the University of Illinois, Chicago. The MPT potential of PPCM was recognised by TOPCAD because it demonstrated significant contraceptive activity as well as anti-infective activity against multiple pathogens.<sup>7</sup>

PPCM, like other polyanions tested to date, is a non-cytotoxic, broad-spectrum antimicrobial agent, with activities against HIV-1, HSV-1, HSV-2, papillomavirus, *N. gonorrhoeae* and *C. trachomatis*.<sup>6</sup> The increased bacterial membrane permeability observed in *N. gonorrhoeae* exposed to PPCM, accompanied by a reduction in the number of recoverable bacteria suggests PPCM directly impacts gonococcal viability. This direct mode of action may explain PPCM-mediated inhibition of *N. gonorrhoeae* in vitro and in the murine model. Other mechanisms may contribute to PPCM-mediated prevention of infection. Many STI pathogens initiate infection by attaching to heparan sulfate or other receptors on the host cell surface. For example, polyanions such as PPCM prevent viral infection by binding to the viral envelope to block attachment to the host cell.<sup>9 10 25</sup> Some *N. gonorrhoeae* adherence and invasion pathways use heparan sulfate glycoprotein receptors,<sup>26</sup> but whether PPCM can prevent *N. gonorrhoeae* infection by blocking these interactions was not tested in our study.

In summary, PPCM formulated into Yaso-GEL continues to show significant promise as an MPT product that is non-hormonal, safe, inexpensive, stable and can be accessed when needed. An intervention with combined gonorrhoea prevention and contraceptive activity is particularly important for at-risk

populations in low- and middle-income countries. Further clinical development is warranted.

**Handling editor** Jonathan Ross

**Acknowledgements** We wish to thank Dr Donald P Waller for the helpful discussions.

**Contributors** AEJ, MBW and BBN contributed to the study concept and design. MP-L and RT contributed to the acquisition of the laboratory data. MP-L and SKB performed the statistical analysis, and SKB prepared the final figures. AEJ, MP-L and RT interpreted the data. MP-L and RT contributed to initial manuscript drafting. AEJ, MBW and BBN were responsible for revisions for intellectual content. All authors approved the final version to be published and agree to be accountable for all aspects of the manuscript.

**Funding** This work was supported by National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, grant R44 HD0902206.

**Competing interests** BBN is the Chief Medical Officer and has equity interest in Yaso Therapeutics. MBW is the President and CEO and has equity interest in Yaso Therapeutics. The patent for PPCM is licensed to Yaso Therapeutics.

**Provenance and peer review** Not commissioned; internally peer reviewed.

**Data availability statement** Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information.

**Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially,

and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

# ORCID iD

Ann E Jerse <http://orcid.org/0000-0002-5118-3172>

# REFERENCES

- World Health Organization. Multi-drug resistant gonorrhea fact sheets. 2021. Available: <https://www.who.int/news-room/fact-sheets/detail/multi-drug-resistant-gonorrhoea>
- Whelan J, Abbing-Karahagopian V, Serino L, et al. Gonorrhoea: a systematic review of prevalence reporting globally. *BMC Infect Dis* 2021;21:1152.
- Cohen MS, Council OD, Chen JS. Sexually transmitted infections and hiv in the era of antiretroviral treatment and prevention: the biologic basis for epidemiologic synergy. *J Int AIDS Soc* 2019;22 Suppl 6:e25355.
- Vincent LR, Jerse AE. Biological feasibility and importance of a gonorrhea vaccine for global public health. *Vaccine* 2019;37:7419–26.
- Young IC, Benhabbour SR. n.d. Multipurpose prevention technologies: oral, parenteral, and vaginal dosage forms for prevention of HIV/stis and unplanned pregnancy. *Polymers*;13:2450.
- Weitzel M, North BB, Waller D. Development of multipurpose technologies products for pregnancy and STI prevention: update on polyphenylene carboxymethylene MPT gel development†. *Biol Reprod* 2020;103:299–309.
- Zaneveld LJD, Anderson RA, Diao XH, et al. Use of mandelic acid condensation polymer (SAMMA), a new antimicrobial contraceptive agent, for vaginal prophylaxis. *Fertil Steril* 2002;78:1107–15.
- Herold BC, Scordi-Bello I, Cheshenko N, et al. Mandelic acid condensation polymer: novel candidate microbicide for prevention of human immunodeficiency virus and herpes simplex virus entry. *J Virol* 2002;76:11236–44.
- Mesquita PMM, Wilson SS, Manlow P, et al. Candidate microbicide PPCM blocks human immunodeficiency virus type 1 infection in cell and tissue cultures and prevents genital herpes in a murine model. *J Virol* 2008;82:6576–84.
- Cheshenko N, Keller MJ, MasCasullo V, et al. Candidate topical microbicides bind herpes simplex virus glycoprotein B and prevent viral entry and cell-to-cell spread. *Antimicrob Agents Chemother* 2004;48:2025–36.
- Escaffre O, Juelich TL, Freiberg AN. Polyphenylene carboxymethylene (ppcm) in vitro antiviral efficacy against Ebola virus in the context of a sexually transmitted infection. *Antiviral Res* 2019;170:104567.
- Escaffre O, Freiberg AN. Polyphenylene carboxymethylene (ppcm) microbicide repurposed as antiviral against sars-cov-2. proof of concept in primary human undifferentiated epithelial cells. *Antiviral Res* 2021;194:105162.
- Kellogg DS Jr, Peacock WL Jr, Deacon WE, et al. Neisseria gonorrhoeae. I. virulence genetically linked to clonal variation. *J Bacteriol* 1963;85:1274–9.
- Jerse AE. Experimental gonococcal genital tract infection and opacity protein expression in estradiol-treated mice. *Infect Immun* 1999;67:5699–708.
- Spencer SE, Valentin-Bon IE, Whaley K, et al. Inhibition of Neisseria gonorrhoeae genital tract infection by leading-candidate topical microbicides in a mouse model. *J Infect Dis* 2004;189:410–9.
- Hillier SL, Moench T, Shattock R, et al. In vitro and in vivo: the story of nonoxynol 9. *J Acquir Immune Defic Syndr* 2005;39:1–8.
- Kulczycki A, Kim D-J, Duerr A, et al. The acceptability of the female and male condom: a randomized crossover trial. *Perspect Sex Reprod Health* 2004;36:114–9.
- Peters A, Jansen W, van Driel F. The female condom: the International denial of a strong potential. *Reproductive Health Matters* 2010;18:119–28.
- Cook RL, Rosenberg MJ. Do spermicides containing nonoxynol-9 prevent sexually transmitted infections? *Sexually Transmitted Diseases* 1998;25:144–50.
- Richardson BA. Nonoxynol-9 as a vaginal microbicide for prevention of sexually transmitted infections: it's time to move on. *JAMA* 2002;287:1171–2.
- CAMI-Health. The MPT product development database. n.d. Available: <https://mpts101.org/>
- Kreisel KM, Spicknall IH, Gargano JW, et al. Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2018. *Sexual Trans Dis* 2021;48:208–14.
- Niforatos JD, Rothman RE. Sexually transmitted infections treatment guidelines, 2021. *Annals of Emergency Medicine* 2022;80:68–70.
- Chappell BT, Mena LA, Maximos B, et al. EVO100 prevents chlamydia and gonorrhea in women at high risk of infection. *Am J Obstet Gynecol* 2021;225:162.
- Park PW, Reizes O, Bernfield M. Cell surface heparan sulfate proteoglycans: selective regulators of ligand-receptor encounters. *J Biol Chem* 2000;275:29923–6.
- van Putten JP, Hayes SF, Duensing TD. Natural proteoglycan receptor analogs determine the dynamics of opa adhesin-mediated gonococcal infection of Chang epithelial cells. *Infect Immun* 1997;65:5028–34.
- Cámara J, Serra J, Ayats J, et al. Molecular characterization of two high-level ceftriaxone-resistant neisseria gonorrhoeae isolates detected in catalonia, spain. *J Antimicrob Chemother* 2012;67:1858–60.
- Unemo M, Golparian D, Sánchez-Busó L, et al. The novel 2016 who Neisseria gonorrhoeae reference strains for global quality assurance of laboratory investigations: phenotypic, genetic and reference genome characterization. *J Antimicrob Chemother* 2016;71:3096–108.