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

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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 gonorrhoea 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.1 The primary site of gonococcal infection is the urethra 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.2 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.3

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.4 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. The addition of contraceptive activity to an STI prevention product is expected to increase its acceptability and actual use. Polyethylene carboxymethylcellulose (PPCM), the active pharmacological ingredient of Yaso-GEL, is a condensation polymer of mandelic acid and an inexpensive topical contraceptive agent. PPCM also has microbicidal activity against sexually transmitted viruses, including HIV, herpes simplex virus (HSV), Ebola virus and SARS-CoV-2. Importantly, PPCM has a good safety profile in preclinical toxicity assays and is not cytotoxic or damaging to epithelial cells. 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. 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 dH2O) containing Kellogg’s Supplement 13 and 12 μM Fe(NO3)3 as described. 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-2 (DPT Laboratories) is a commercially available spermicidal gel that contains 2% nonoxynol-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% CO2, at 37°C on supplemented GC agar. CC 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. 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 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 20–22-hour incubation and suspended in GC broth (GCB) to a concentration of 107 colony-forming units (CFUs) per millilitre. Ten microlitres of each suspension (ca. 105 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 (OD600) was adjusted to 0.08. Filtered suspensions were then incubated in T25 tissue culture flask at 150 rpm for 3 hours at 35.5°C, after which the OD600 of the cultures was adjusted again to 0.08 (−10 CFUs/mL). The suspension was then diluted 1:50 in GCB, and 100 μL (−103 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% CO2, 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

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Susceptibility profile</th>
<th>MIC (µg/mL)</th>
<th>Agar dilution</th>
<th>Microtitre plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA1090*</td>
<td>STMa</td>
<td>25</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>MS11</td>
<td>STMa</td>
<td>100</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>H041 (WHO X)²</td>
<td>PENr, TETr, AXr, CIPr, CFXr, CROr, STMr</td>
<td>25</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>F89²</td>
<td>Penr, TETr, CFXr, CROr, STMr</td>
<td>≤5</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>WHO Ft</td>
<td>susceptible</td>
<td>≤5</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>WHO Gt</td>
<td>PENr, TETr, CIPr</td>
<td>≤5</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>WHO Kt</td>
<td>PENr, TETr, CIPr, CFXr</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>WHO Lt</td>
<td>PENr, TETr, CIPr, CROr</td>
<td>100</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>CONUS-9542t</td>
<td>TETr, CIPr</td>
<td>100</td>
<td>Not determined</td>
<td></td>
</tr>
<tr>
<td>CONUS-3668t</td>
<td>PENr, CIPr</td>
<td>≤5</td>
<td>Not determined</td>
<td></td>
</tr>
<tr>
<td>CONUS-6364t</td>
<td>PENr, CIPr</td>
<td>25</td>
<td>Not determined</td>
<td></td>
</tr>
</tbody>
</table>

*Commonly used laboratory N. gonorrhoeae strains. Multidrug-resistant, ceftriaxone-resistant N. gonorrhoeae strains. TUS isolates; from the USU GC Resistance Repository and Reference Laboratory isolated in 2017 (CONUS-9542), 2019 (CONUS-3668) and 2014 (CONUS-6364). AZL, azithromycin; CFX, cefixime; CIP, ciprofloxacin; CRO, ceftriaxone; HLR, high-level resistant; I, intermediate susceptible; LLR, low-level resistant; N/A, not available; PEN, g, pencillin G; PPCM, polyethylene carboxymethylcellulose; R, resistant; STM, streptomycin; TET, tetracycline.
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 fluorescence 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 percent 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 ≤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 10⁷ 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 microbicidal methylcellulose.15

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 vitro efficacy of 2 mg/mL against *N. gonorrhoeae* strain MS11, which is 10× 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.16 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≤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 0.25 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).
GC agar


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 10⁵ 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.

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. 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. Early in vitro and limited clinical studies suggested that N-9 might offer some protection against gonorrhea as well as contraception. Unfortunately, the cytotoxic effects of N-9 can actually damage host genital epithelial cells and may even increase the risk of HIV infection. 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. 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. Gonorrhea is particularly problematic not only because of significant global incidence and serious health sequelae, but worsening antibiotic resistance has severely limited treatment options. Only one vaginal product, a recently marketed vaginal contraceptive, is in clinical trials for N. gonorrhoeae and C. trachomatis prevention.

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. We therefore compared the pH of PPCM prepared in GCB at concentrations of 1.56 µg/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 µg/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.

(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.
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=0.04 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 over time 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 log10 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.
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.7

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*.6 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.9 10 25 Some *N. gonorrhoeae* adherence and invasion pathways use heparan sulfate glycoprotein receptors,26 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 gonorrhea prevention and contraceptive activity is particularly important for at-risk populations in low- and middle-income countries. Further clinical development is warranted.

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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.

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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.

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