Biology of Reproduction, 2020, 103(2), 299–309
doi:10.1093/biolre/ioaa087
Contraceptive Special Issue
Advance Access Publication Date: 29 May 2020

OXFORD

Contraceptive Special Issue

Development of multipurpose technologies products for pregnancy and STI prevention: update on polyphenylene carboxymethylene MPT gel development†

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†Grant Support: This work was supported by the National Institutes of Health under grants R42 AI069659 (M.W.), R43/44 AI084225 (M.W.) and R44 HD092206-01 (M.W.).

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Conference Presentation: This work was presented in part at the 2019 NICHD Contraception Development Meeting October 3-6, Houston TX.

Received 23 February 2020; Revised 18 May 2020; Editorial Decision 21 May 2020; Accepted 27 May 2020

Abstract

Current modern contraceptives rely heavily on the use of hormones. These birth control drug products, including pills, patches, injections, and IUDS, have been extremely beneficial to millions of women and their families over the past 50 years. But a surprisingly high number of women abandon such modern methods, many because they cannot tolerate the side effects and others because they have medical issues for which hormonal methods are contraindicated. In addition, modern hormonal methods are simply not available to many women. The extent of this problem is steadily becoming more apparent.

We present the case for developing simple nonhormonal vaginal products that women can use when needed, ideal products that are multipurpose and offer both contraception and sexually transmitted disease protection. Gel-based vaginal products are particularly well suited for this purpose. Gels are easy to use, highly acceptable to many women, and can be safely formulated to enhance natural vaginal defenses against infection. However, the development of a new chemical entity for this application faces significant technical and regulatory hurdles. These challenges and our solutions are described for polyphenylene carboxymethylene (PPCM), a novel topical drug in a vaginal gel nearing human clinical trials. We have advanced PPCM from benchtop to IND-enabling studies and provide a brief description of the complex development process. We also describe a simple lab assay which can be used as a biomarker for contraceptive activity to enable pharmacodynamic studies in vaginal contraceptive development, both preclinically and in early human clinical trials.

Summary sentence

Nonhormonal contraceptive and microbicidal multipurpose drug products are needed to fill a gap in available products: development of polyphenylene carboxymethylene, a candidate vaginal gel is described.

Key words: acrosome, contraception, hormone, semen, seminal plasma, sperm, sperm motility, toxicology, vagina, vaginal epithelium, microbicide, multipurpose.

Introduction

More pregnancy prevention methods are needed

Around 40% of pregnancies worldwide are unintended and 45% of pregnancies in the USA continue to be unplanned—higher than in other industrialized countries [1, 2]. This persists in spite of the development of highly effective contraceptives. The long-acting reversible contraceptives (LARCs) have been particularly successful and are likely responsible for recent reductions in unplanned pregnancies among teens [1]. Oral contraceptives continue to be the most popular reversible method. Yet, overall contraceptive use has not significantly increased [3]. Use of these hormonal drug and device products is limited by health concerns and side effects, availability, costs, and access to medical services [4–6]. In the USA, at least 19 million women who depend on publicly funded contraception services live in contraceptive "deserts." These are countries that lack accessible health centers which provide a complete range of contraceptive services [7]. Even more distressing is the "condom migration" that occurs with hormonal contraceptive users. They can be less likely to use condoms compared with women who use other contraceptive methods and may be more susceptible to sexually transmitted infections (STIs) [8–10]. Additionally, many U.S. women who previously used hormonal birth control methods are now at risk of pregnancy because they use nothing, primarily because of dissatisfaction with or distrust of hormones [5, 11]. The situation may be even more worrisome in developing countries. A recent comprehensive survey of 36 low- and middle-income countries found that the use of long-acting modern methods remains consistently low. Among women with an unintended pregnancy who last used a long-acting modern method, 40% had discontinued the modern method because of side effects [12]. Not only do many women distrust hormonal birth control methods, but also some women believe that any hormonal method may have postfertilization activity and is actually an abortifacient [13].

This concern about health risks associated with hormonal contraceptives is shared by many family planning professionals. Interactions with other drugs, effects of obesity, and chronic disease conditions are all of concern [14]. Recently, an increased breast cancer risk has been linked to hormonal contraception [15]. While the risk is presumably small, it contributes to women's concerns.

The need for more contraceptive product choices is an accepted reality [16]. When a greater variety of contraceptives are made available, more women use contraception. This has been shown globally: in countries where only a single method is made available, only about 10% of women will practice contraception. This increases linearly up to 70% contraceptive use when six methods are available [17]. Obviously, no single method is equally acceptable to all women. Method acceptability varies with partner and family relationships, age, personal preferences, cultural influences, as well as perceived safety and efficacy [16]. Many women may prefer a nonhormonal method that can be easily accessed only when needed and is not absorbed into the body [2, 18, 19]. Yet, with the exception of detergent spermicides, nearly all woman-controlled contraceptives available today depend on continuous systemic hormonal activity or intrauterine devices.

STI prevention for women is lacking

Women who engage in intercourse may be at significant risk for both an unplanned pregnancy and an STI. Ideally, a preventive method will provide dual protection and be woman-controlled. The worldwide incidence and prevalence of STIs are growing [20–23]. In the USA, the reported incidence rates of chlamydia and gonorrhea have grown dramatically, and one in six adults has genital herpes [20, 22]. Drug resistant strains of gonorrhea are continuing to appear, both globally and in the USA, foreshadowing a time when many people may struggle to find a cure [24]. Clearly, the field of reproductive health needs more solutions to help turn the tide of increasing STIs and unplanned pregnancies. We must develop a variety of multipurpose prevention technologies (MPTs) drug products to provide comprehensive protection from both unwanted pregnancy and a range of STIs. This may be particularly important for young women who may be more anxious about accidental pregnancy than acquiring an STI [25–29]. Ideally, this broader protection would be provided by a single multipurpose MPT product [30].

Materials and methods

Ethics approval and consent to participate

The sperm study was conducted in accordance with principles for human experimentation as defined in the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines and approved by the relevant institutional review boards. Informed consent was obtained from all participants.

Animal studies declaration

All animal studies were conducted following recommendations for optimal animal welfare with the approval of the Office of Laboratory Animal Welfare, in AALAC approved facilities.

Multipurpose prevention technologies

MPTs are an innovative class of products that deliver various combinations of human immunodeficiency virus (HIV) prevention, other STI prevention, and contraception. While not labeled as MPTs, some older vaginal products were potential MPTs. Early, in vitro and limited clinical studies suggested that the spermicide nonoxynol-9 (N-9) might offer some STI protection as well as contraception. In fact, significant protection against both chlamydia and gonorrhea infections was demonstrated in women who used a vaginal sponge impregnated with N-9 [31]. Unfortunately, the cytotoxic effects of N-9 and potential to facilitate HIV infection have discouraged use and further development [32, 33]. The female condom, which could potentially be an effective MPT, has had very limited acceptance [34, 35].

Current development

In the last decade, this unmet need for woman-controlled dual protection has become more widely recognized and has evolved into an active pipeline for the development of MPTs [26, 28, 29, 36–39]. The intravaginal ring (IVR) is a promising MPT in active clinical development. IVRs offer sustained drug release, which avoids some of the compliance issues of coitally dependent use, but they are not a panacea. Acceptability may be limited by cost, user distrust of hormonal contraception, menstrual irregularities, poor fit leading to expulsions, and difficulty with insertion and removal [40–42]. In addition, user dissatisfaction can cause many women to remove the ring and lose protection [43]. Male partner resistance can also be a barrier to successful IVR use, particularly for women who suffer from power inequities in sexual relationships [40, 41].

Several new coitally dependent methods are under development as well, including gels, films, and tablets [30]. However, such as IVRs, nearly all these vaginal products depend on hormonal contraceptive action. In addition, the STI component of these MPTs has focused on HIV prevention, primarily by the use of a vaginal microbicide, with relatively little attention to other important STIs. While the extreme morbidity and mortality linked with HIV is widely feared, other STIs—particularly HSV, *Neisseria gonorrhoeae* (Ng), and Chlamydia trachomatis (Ct)—pose a greater infection risk to many women and their partners, particularly young women in the developed world [44, 45]. While treatable and sometimes curable, they can also cause significant disease, permanent infertility, and can occasionally be fatal. Efforts to develop vaginal microbicides have essentially ignored these important infections. Vaccine development for all STIs is ongoing, but except for Human Papilloma Virus and Hepatitis B, effective vaccination for other STIs is not imminent [25]. A shift in emphasis from HIV protection to broader STI protection with focus on gonorrhea and chlamydia infections is long overdue, ideally in combination with effective contraception.

Barriers to MPT development

Why are not more companies working on new vaginal MPTs? Simply put, the fastest path to introducing a new contraceptive is to make incremental changes to existing drug products. Highly effective LARC's and contraceptive pills are available and widely used. Small changes to existing methods, primarily hormone-based methods and condoms, require minimal clinical trials and have a clear regulatory pathway, which reduces the time and cost required to supply a well-established market. This is more attractive to drug companies.

All coitally dependent methods face much greater development challenges. Efficacy rates are difficult to assess—primarily because many women find it difficult to adhere to a method that must be used consistently and correctly with every act of intercourse. Recent clinical trials have highlighted this problem. In the case of HIV microbicide trials, the actual effectiveness has been significantly underestimated due to multiple factors, including pervasive adherence issues, inaccurate reporting by study subjects or frequent nonuse, as well as HIV acquisition via anal intercourse or intravenous drug use [46–48]. In a recent hormonal contraceptive study, misreporting occurred in 27% of participants who had used but would not admit to using backup contraception [49]. In retrospect, these difficulties are not unexpected, given the challenges faced by the women enrolled in these HIV prevention trials:

"Imagine getting a group of largely poor women in a developing country, whose role is still largely to marry, submit uncomplainingly to sex and support their family, to agree to squeeze goo every day for a year (or every time they think hubby is going to want sex) into their vaginas, do it despite the disapproval of conservative moms and suspicious spouses, report regularly to a clinic for checkups, and answer impertinent questionnaires about their sex lives to researchers, when they've been told that half of them are getting a placebo anyway." … … Gus Cairns [50]

In spite of these frustrations, subsequent data analysis has uncovered some important protective effects against both HIV and HSV in women who demonstrated the highest compliance [51–53].

Simply enrolling women in contraceptive trials is equally problematic. Like HIV prevention trials, contraceptive testing requires large numbers of participants who must participate for 6–12 months. In addition, participants are being asked to risk becoming pregnant. This is not a small issue. Women who become pregnant are forced to choose either termination or carrying the pregnancy to term. Since highly reliable birth control methods are available to them, it is reasonable to assume that many women in these trials are willing to accept a pregnancy and they may not be highly motivated to use the assigned method. This may be particularly true for women who are planning to increase their family size [54, 55]. This attitude was confirmed by a recent online survey we conducted asking 504 heterosexual women if they would be willing to try a new contraceptive gel and how they would feel if they got pregnant. Sixty percent of nonmothers responded that it would be a serious problem or even "disastrous." In contrast, only 35% of the mothers were as concerned.

In addition to imperfect use and misreporting, the frequency of intercourse, age, fertility, methodological problems, and even investigator bias—all contribute to the inaccuracy of effectiveness measurements [56]. An additional problem is that even with "perfect use," nonsystemic vaginal products are likely to have inherent method failures. Unlike vaginal methods that release systemically absorbable contraceptive substances, the effectiveness of products that act only in the vagina (gels, films, foams, and suppositories) depend on their ability to coat vaginal/cervical tissues immediately after application and mix with genital secretions postcoitally. Vaginal anatomy is highly variable [57], and it is unlikely that any vaginally applied product will always provide perfect coverage and/or achieve complete inactivation of the sperm. All these uncertainties contribute to the wide variation in effectiveness rates that have been reported for vaginal spermicides [58, 59]. Even though women who are reliable users of coitally dependent contraceptive methods can expect a higher effectiveness than is reported in most clinical studies, efficacy will always be lower compared with methods that offer continuous protection. But efficacy is only one determinant of contraceptive choice, and women may reject highly effective methods because of other concerns [59]. Interestingly, a low-efficacy method may be chosen even by women who do not ever want to become pregnant. A recent U.S. study of women at risk of pregnancy found that a low-efficacy method was chosen by 20 % who "never in the future" want to become pregnant, by 27% who wish to "delay pregnancy," and by 29% who are "not sure" [60].

Development of novel coitally dependent products has also been hampered by the lack of established preclinical and clinical protocols. Work on vaginal HIV prevention products has spawned a plethora of recommendations for preclinical testing and many of these are applicable to any vaginal product [61]. This has resulted in a constantly changing regulatory environment, which complicates the development path even more [36, 62, 63]. Lack of a reliable animal model for contraception has added to the difficulty. Size, shape,

secretions, pH, reproductive cycle, and microbiome of commonly available animal models are poorly matched to the human vagina [64, 65]. Consequently, formulations designed to take advantage of the natural protective barriers and function of the human vagina cannot be adequately studied in current animal models.

Vaginal contraceptive studies are also limited by the lack of established biomarkers for spermicidal activity [61]. Such biomarkers could be used to verify activity and proper formulation development both preclinically and in early Phase I human testing, before engaging in additional long-term and difficult efficacy trials where women are at risk for pregnancy. Traditionally, sperm activity has been measured by observing the numbers of progressive motile sperm. This has assumed that such sperm have fertilization potential. The actual methodology is labor-intensive and requires a ready supply of mature, healthy sperm, along with skilled microscopy, all of which is time sensitive. In clinical trials with women not at risk of pregnancy, cervical mucus must be collected soon after coitus and immediately examined microscopically (Sims-Huhner test) [66, 67]. Not only is such postcoital testing labor-intensive and time-sensitive for the experimental subjects, the laboratory and the investigator, but it also has poor reproducibility [66]. This contrasts with microbicide development work for STI pathogens which have well established precise in vitro assays for activity on samples that can be easily obtained from the vaginal vault, transported, stored, and processed with fewer time constraints [68]. Such anti-infective efficacy can also be assessed with numerous validated animal models [69].

The family planning community inadvertently discourages coitally dependent MPT development and has an innate bias in favor of hormonal-based contraceptives. Because they are not coitally dependent, these methods are more effective and much easier to use. But easier for the woman or for the physician/provider? The provider can simply write a prescription or give an injection or implant a device, and most adherence uncertainties are avoided. But for many women, these drugs and devices are unacceptable, and efficacy is only one determinant of contraceptive choice. Highly effective contraceptive methods may be rejected because of other concerns. Hormone-based methods require medical services that are at best inconvenient, have unpleasant side-effects, are expensive, or may not even be available. Furthermore, some women do not want continuous protection. Not all women are in a steady relationship or have infrequent intercourse, and thus are not willing to use long-acting drug products. The availability of a method that can be used only when needed is an important option for many women [2, 18, 19].

Another difficulty for developers is that the market for a vaginal contraceptive is difficult to gauge since few comparable products are currently on the market, whereas the market for a new hormonal contraceptive or new IUD is easily estimated. Yet, we know that women will use vaginal products. Women in developed economies already know and use vaginal drug products and lubricants. Nearly 29 million units of personal lubricants—valued at over $1 billion—were sold in America last year through retail outlets, not including online and boutique sales [70]. Twenty-seven million units of OTC vaginal yeast treatment products were sold in 2018 through retail outlets [71]. Proper formulation, which includes aesthetics as a key criterion, and consumer marketing are critical and effective driving forces for adoption of a vaginal product.

An MPT dual purpose vaginal product is expected to have greater acceptability and compliance compared with a single purpose vaginal contraceptive or microbicide. It will be an attractive option for women who are not only concerned about their STI risks but are even more concerned about pregnancy prevention [25–29, 39]. Furthermore, women who seek contraception in addition to STI protection are likely to have greater support from partners, family, and community [72–77]. All of these factors will improve compliance both in clinical trials and more importantly, use in the real world.

The case for vaginal gels

Vaginal gels can be engineered to maintain, improve, and take advantage of natural protective mechanisms while providing a versatile vehicle for active contraceptive and anti-STI activity [78]. Natural protection from pathogens is primarily due to a hostile fluid environment maintained by a healthy microbiome and mucus. A *Lactobacilli* dominant microbiome produces an acidic environment that is very effective in killing and inactivating most STI pathogens, including both viruses and bacteria as well as inactivating spermatozoa [79].

Secreted mucus is an important contributor to prevent both disease and pregnancy. Vaginal epithelial cells provide a transudate to the vaginal vault environment, whereas the mucus layer is derived from the cervical secretions, which varies during the reproductive cycle. Mucus can act as a barrier to spermatozoa ascending the reproductive tract and pathogens accessing tissues. Semen deposition neutralizes and dilutes this protective vaginal environment. A vaginal gel can help counteract these effects by maintaining both the acidity and the viscosity of vaginal fluids [80, 81].

Natural vaginal fluids also provide lubricity, reducing vaginal abrasions and tears from intercourse, which can readily provide a portal of entry for pathogens. Vaginal gels can amplify this property with a formulation that enhances the sexual experience for both partners while reducing susceptibility to STIs, as well as help overcome adherence issues [82].

Gels also have the advantage that they can be designed to optimize coating of the entire vaginal epithelium. This can minimize the potential for reduced efficacy due to the large variation in shape and sizes of the vagina. Gels can also be formulated to maximize mucoadhesion to ensure that the gel is retained at the most critical time—during and after sexual intercourse. Gels may be active immediately upon insertion into the vagina and are slowly expelled after intercourse by natural vaginal secretions [83]. In contrast, long-acting methods generally release active ingredient(s) slowly and must be inserted well ahead of intercourse. They must provide sustained release to maintain effective drug levels and may create as yet unknown negative side effects with extended exposure.

Multiple acceptability surveys of preferred vaginal formulations have found support for odorless and colorless gel products and can be an important guide to the development of new vaginal gel products [84]. Recent studies have provided important information regarding user preferences for the physical and aesthetic properties of gel formulations and can be used to guide MPT formulation composition [85, 86].

Development of a candidate MPT—polyphenylene carboxymethylene gel

Polyphenylene carboxymethylene is contraceptive and microbicidal

Polyphenylene carboxymethylene (PPCM sodium salt) was originally developed as a potential MPT by the TOPCAD research group at Rush University. PPCM is an anionic mandelic acid condensation polymer that may provide excellent MPT protection [87–92]. It differs from other polyanion candidate products in several important

ways. First, PPCM is not sulfated/sulfonated and thus is structurally distinct from Pro 2000, PSS (polystyrene sulfonate), Carragard, and CS (cellulose sulfate). Unlike these sulfated/sulfonated polyanions, PPCM does not lose effectiveness in the presence of seminal plasma [93–96]. Unlike N-9 and CS, PPCM does not damage epithelial surfaces [97]. Finally, in contrast to the other sulfated/sulfonated polyanions, PPCM does not reduce levels of SLPI, an important regulator of innate immunity that may protect the host from excessive/dysregulated inflammation typical of infectious diseases [98–100]. These factors may have contributed to the clinical failures of these polyanions in Phase III microbicide trials.

PPCM is active against multiple sexually transmitted pathogens—both viral and bacterial. Extensive preclinical testing has demonstrated that PPCM is active against HIV-1, HSV-1, HSV-2, papilloma virus, ebola virus, Ng, and Ct [89, 90, 92, 93, 101, 102]. PPCM, like other polyanions, prevents viral infection by binding to the viral envelope to prevent attachment to heparan sulfate (or similar) receptors on the host cell [103]. Glycosaminoglycans such as heparan sulfate are believed to be important for host cell attachment and infectivity of many other STI pathogens as well, including Ng and Ct [104–107]. Since these receptors are thought to be highly conserved, the development of pathogen resistance to PPCM is unlikely [105, 107, 108]. This is particularly important for future Ng prevention technology, where rapid and continuous evolution of antibiotic resistance is a major challenge [109].

PPCM is also innovative because, unlike most currently available spermicides, it has a noncytotoxic contraceptive mechanism. PPCM causes a premature loss of the sperm acrosome (PAL), a critical organelle required for successful sperm–oocyte interaction. This contraceptive activity may be caused by induction of PAL through dysregulation of Ca^{2+} signaling [87, 88, 110]. The same mechanism may be partly responsible for the multiple activities of this contraceptive microbicide. Infection of target cells by HSV and HIV is associated with Ca^{2+} signaling in the target cells [88, 111]. In addition to causing PAL, PPCM inhibits hyaluronidase and acrosin, the acrosomal enzymes needed for fertilization. Inhibitors of these enzymes are contraceptive in the rabbit model [112, 113]. Interestingly, PPCM has little or no effect on human spermatozoa motility [92].

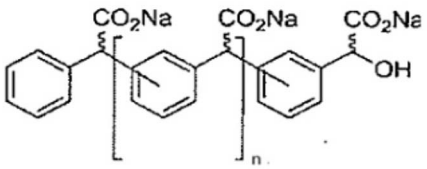
PPCM is an effective contraceptive in the rabbit model. Fertilization of oocytes was reduced by >90% when spermatozoa were preincubated with 5 mg/mL PPCM before artificial insemination. A 4% PPCM gel completely prevented conception when placed vaginally before artificial insemination [92].

Synthesis and characterization

PPCM is a polyanion with an average (Mw) molecular weight = 3500–5000 (Figure 1).

As a polymer drug, PPCM faces a complex regulatory strategy as it does not fit into either of the two main molecular categories of drugs, namely small molecules and biologics. PPCM was first licensed with a lab scale synthetic method after early preclinical studies indicated anti-infective and contraceptive activity against HIV, HSV, Ng, Ct, papilloma virus, trichomoniasis, and spermatozoa [92]. However, no analytical methods had been developed and the detailed molecular structure was in question. Consequently, the analytical methods used to characterize the molecular structure were developed de novo. The molecular structure was finally determined using 900-mHz NMR.

All analytical methods were developed and validated for the measurement of the stability, quantity, identity, and purity of PPCM.



PPCM Na Salt

Molecular Formula: C8H6O2Na(C8H5O2Na)nC8H6O2Na

Figure 1. Molecular formula: $C_8H_6O_2Na(C_8H_5O_2Na)nC_8H_6O_2Na$.

A bioassay to determine systemic absorption, requested by FDA, was developed using LC-MS/MS techniques and validated in rat and rabbit plasma and vaginal tissue with an LLOQ of 5 ng/mL.

The scale up and commercial production of PPCM have also been challenging. To our knowledge, there are only two approved soluble, active polymer drugs: Copaxone (Glatiramer) and Voluven (hydroxyethyl starch) [114]. Thus, few CROs who support drug startups have had any experience with a new, active polymer drug (as opposed to excipients) and polymer producers are often not qualified to produce these drugs or not familiar with drug quality requirements.

The manufacturing method for the API was optimized including the development of quality assurance procedures. The manufacturer successfully passed a QA audit, and a quality contract was executed. A production batch of API was produced under cGMP and put on a stability program under ICH guidelines. At T = 12 months, PPCM remains within specifications and is stable. Two forced degradation studies, including one performed according to the FDA and ICH guidelines, demonstrated that PPCM degrades simply to shorter oligomers with no other detectable break down products. PPCM in aqueous solution showed degradation under high oxidation (3% hydrogen peroxide, 7 days, 13.3% degradation) and slight degradation at low pH (1 N HCl, 6 days, 0.1% degradation). PPCM is extremely stable under ambient storage conditions and in aqueous solution. Laboratory produced batches from 2002 to 2008 were also analyzed and found to be stable. Table 1 includes our current specifications for PPCM.

Formulation development

The challenge is to develop a formulation that maximizes the inactivation of spermatozoa in the complex milieu of the vaginal environment, with special attention to vaginal pH, secretions, cervical mucus barrier, microbiome, semen composition, and shear/mixing action during coital activity. A variety of novel mucoadhesive formulations with 4% PPCM Na Salt were developed based on optimum viscosity, pH, rheological characteristics, and osmolality. The effects of dilution on characteristics were considered as well; the gel should be miscible with vaginal secretions, cervical mucus, and seminal fluid without leaking out during intercourse. To assess this, a progressive 8-h in vitro study of PPCM gel release into both simulated vaginal fluid and simulated semen was conducted. A wide variety of excipients were considered based on literature assessments for specific properties to enhance the formulations with desired mucoadhesive and releasing characteristics, including HPMC (K4M, K100 or E50), Xanthan and/or Guar Gum, Carbopol 940, chitosan, Poloxamers. HEC (Natrasol 250 HR), and Na CMC.

Table 1. Selected Specifications of Batch BPR-18-12-B1-19 PPCM sodium salt

Property	Specification	Test results	Test method
Appearance	White to off-white powder	Pass	Visual
Identification			
IR spectrum	Conforms to structure	Pass	FTIR
pH (5% in water)	FIO	7.13	PPC107
Mw range	3500–5000	3835	HPLC-GPC with SEC
Polydispersity (Mw/Mn)	<1.70	1.40	HPLC-GPC with SEC
Purity			
HPLC—ion exchange (IEX)	Target—95–105% AUC	99%	HPLC-IEX
HPLC—impurities by RRT	Report results	<LOQ (0.05%)	WPT/2068-182

Six different 4% PPCM sodium salt formulations were prepared: altering methods of preparation as needed based on compatibility and solubility data. Both Carbopol/Polycarbophil and Xanthan gum/HPMC formulations showed better mucoadhesive strength compared with the marketed product Gynol II with an acceptable pH profile. Our final formulation is comprised of deionized water, PPCM sodium salt (4% w/w), HPMC K100, xanthan gum NF, glycerin, methyl paraben, propyl paraben, and pH adjusters (lactic acid and sodium hydroxide, as needed). The resulting gel, at T = 3 months under controlled stability (25 °C/60% RH), has a viscosity of 3.97×10^7 mPa-s, a pH of 5.4. The osmolality (1020 mOsm/kg) is below the WHO maximum acceptable level for vaginal lubricants [115] and is also below the level shown to cause epithelial disruption in a 3D model [116]. Osmolality would be further reduced in the presence of genital secretions after vaginal application.

Optimized formulations were manufactured under GLP to conduct a stability study as per ICH guidelines and to provide test articles for GLP toxicology studies. Specifically, gels at 0, 1, 4, and 10% concentrations were held at 25 °C/60% RH and 40 °C/75% RH for 6 months. Samples were analyzed for appearance, viscosity, pH, and assay at 1, 3, and 6 months.

Preclinical toxicology testing

Table 2 lists results of the preclinical toxicology IND-enabling studies required by the FDA for a new chemical entity, which is intended for contraception and disease mitigation.

Development of a biomarker for PPCM contraceptive activity

Since PPCM does not directly affect motility, postcoital testing for sperm motility cannot be used to assess sperm inactivation. Early work on PPCM identified PAL as a likely mechanism of action, and an acrosome staining (AS) method has been used to assess acrosomal loss as a surrogate marker of sperm inactivation. But like postcoital testing for motility, this method is technician-dependent, tedious, and complicated [92]. A more convenient measure of sperm activity would be useful for in vitro preclinical studies and could potentially serve as a biomarker to assess the contraceptive effectiveness of PPCM in the vagina. Both the contraceptive and anti-infective actions of a nonsystemic vaginal product occur in the vaginal vault and may be limited by uneven distribution and leakage. In addition, fluids from the vagina, cervix, or seminal fluid contain complex proteins and other substances that may reduce effectiveness when mixed with a vaginal product [96, 117–119]. A better biomarker for sperm activity could help us learn more about these effects in early clinical testing of ex vivo vaginal samples as well as in preclinical studies. This in turn can guide the design of subsequent proof of concept clinical testing. We can make better decisions about subsequent clinical trial design if PPCM gel efficacy can be determined without risk of pregnancy.

An inexpensive, user-friendly, commercially available hyaluronan binding assay (HBA) may be such a biomarker. Hyaluronan Binding Assay (HBA) is a validated test to assess the functional examination of human sperm. Only mature sperm are able to bind to the hyaluronan contained in the zona pellucida of the mature oocyte, which is the initial step in fertilization. Thus, anything which prevents this binding step will prevent fertilization [120]. Multiple studies demonstrate that the fraction of motile sperm that bind to the hyaluronan substrate in the HBA is directly correlated with numerous parameters of sperm health—including greater progressive motility, lower DNA fragmentation, reduced sperm agglutination, normal overall morphology, as well as an intact acrosome [121–124]. The HBA has been extensively evaluated in the context of assisted reproductive technology and has been used to isolate and select healthy sperm for in vitro fertilization [125, 126]. Sperm—either washed or in neat semen—are placed on the slide, and the ratio of bound to unbound sperm is easily determined microscopically. Since HBA reliably identifies the fraction of mature healthy sperm with fertilization potential in a washed sperm or semen sample, the assay will also measure reduction in the fraction of healthy sperm due to inactivation by a contraceptive.

We have conducted preliminary studies in vitro using HBA to assess PPCM contraceptive effectiveness (MS in preparation). HBA binding was validated against PAL sperm inactivation measured with AS [127]. Sperm incubated with increasing concentrations of PPCM exhibited a linear decrease in the fraction of viable sperm, measured by both HBA and AS. While the EC50 ~ 0.1 µg/mL with both methods, HBA detected inactivation of a much larger fraction of the total sperm population compared with the AS technique. Up to 80% of the total sperm were inactivated (unbound) shown by HBA after exposure to PPCM. This contrasts with about 25% inactivated by PAL and identified by AR staining. The superior sensitivity of HBA is also seen with the positive control (ionophore) (Figure 2).

HBA may be suitable for measuring the contraceptive effectiveness of other vaginal products preclinically for studies that assess sperm maturity and viability. It may prove to be particularly useful for early phase clinical trials to assess spermicidal activity in the vagina before and after application of a vaginal contraceptive product—both pre- and postcoitally. Thus, HBA may provide relevant data for human sperm inactivation potential of PPCM and lead to the validation of an important assay to assess additional PPCM

Table 2. Comparison of sperm inactivation by PPCM measured by both an HBA and AS

Completed		
Genotoxicity	Study description	Study results
GLP Reverse Bacterial Mutation Assay (AMES), Bioreliance	Five strains Salmonella and *Escherichia coli* plated w/PPCM up to 5000 µg/mL; evaluated for toxicity and mutations	PPCM was nontoxic to selected strains. PPCM is not mutagenic in AMES assay
GLP Rat Lymphoma Assay, Bioreliance	PPCM solution of 1.95–500 µg/mL plated with L5178/TK+/− mouse lymphoma cells w/or w/o S9 metabolic stimulator	PPCM is not mutagenic
GLP Rat Micronucleus Assay, Bioreliance	Male SD rats, 25, 50, 100, and 200 mg/kg, 10 mL/kg IV, once. Groups harvested 24 and 48 h	No significant increase in the incidence of micronuclei at either time point. No significant reduction in %PCE compared to control; indicates PPCM is not cytotoxic
Safety toxicology		
Non-GLP acute oral toxicity rats MB research	12 M/F Sprague Dawley rats dosed with either 1000 or 2000 mg/kg PPCM	A single dose of either 1000 or 2000 mg/kg had no observed toxicological effect
Non-GLP acute IV toxicity rats—MB research	6 M/F Sprague Dawley rats dosed w/single injection 10, 50, or 100 mg/kg PPCM in water solution	No toxicological effects seen at 10 mg/kg. Some distress observed at 50 and 100 mg/kg on day 1. No distress days 2–14. No deaths occurred
Non-GLP 3-day Labial Irritation—rabbits—Sinclair Research	24F New Zealand Whites; 0.2 mL of either vehicle control, 4, 10, or 30% PPCM gel applied to labia; scored pre- and postdose	PPCM-gel was well tolerated with concentrations up to 300 mg/mL
Non-GLP tissue distribution/route of excretion of 14C-PPCM—SRI Intl.	Vaginal application of 0.4 and 4% 14C-PPCM with sampling of blood at 1, 4, 8, and 24 h	On average, about 1–2% of the total radioactive dose was in the blood for the 4.0% group, and <1% for the 0.4% group at each time point
GLP 28-day vaginal/labial/penile irritation in rabbits w/PK Tk and 14-day recovery	Saline, 1, 4, and 10% plus vehicle control doses 1.0 mL 28 consecutive days. 2 animals/grp allowed to recover for 14 days prior to sacrifice. Pk samples collected @ 6 times day 1 and day 28. Bioassay by LC/MS-MS and data analyzed Necropsy and histopathology	No clinical symptoms in either males or females. No test article-related early deaths; no notable test article-related clinical observations; no changes in body weight, food consumption, clinical chemistry, hematology, coagulation, or urinalysis parameters; and no gross pathology findings or organ weight differences in males or females. Low drug concentrations were detected in plasma of both males and females. No penile irritation and only mild to moderate vaginal irritation was observed in a dose dependent manner. Systemic NOAEL = 10% and vaginal NOAEL = 4%
Non-GLP DRF Oral SD rats	A dose range finding study w/7-day repeat dose (8M/8F) Sprague Dawley rats at 1000 and 2000 mg/kg. A single bioassay sample taken 24-h post final dose	No mortality during this study. There were no PPCM sodium salt-related clinical observations, changes in body weight, coagulation parameters, or gross pathology findings
GLP 28-day oral toxicity in SD rats w/Pk Tk and 14-day recovery	PPCM aqueous solution. Doses of 500, 1000, and 2000 mg/Kg plus control group. 15M and 15F per group, with 5/gp recovering for 14 days. Bioassay samples pulled 6 times Day 1, Day 28, and Day 42	Ongoing

formulations or other sperm inactivating compounds in the human vaginal milieu.

Summary

There is clearly a need for an MPT that provides both STI protection and a nonhormonal contraceptive choice. Development of an MPT that is woman-controlled, administered vaginally, not systemically absorbed, and available OTC without medical services will be an important choice for dual protection. This is particularly true for women who want an "on-demand" method, who need only episodic protection, women who cannot tolerate side effects of systemic and/or hormone-based products, or for whom the medical services needed to obtain these methods are simply not available.

A gel formulation is a logical candidate for vaginal application. We describe the development of a candidate MPT product utilizing PPCM, a novel polyanion with noncytotoxic contraceptive and anti-infective activity. The formulation is designed to maximize the inactivation of spermatozoa in the complex vaginal milieu.

Development of novel nonhormonal coitally dependent contraceptive drug products is particularly difficult and faces resistance from several quarters. However, the undertaking is worthwhile

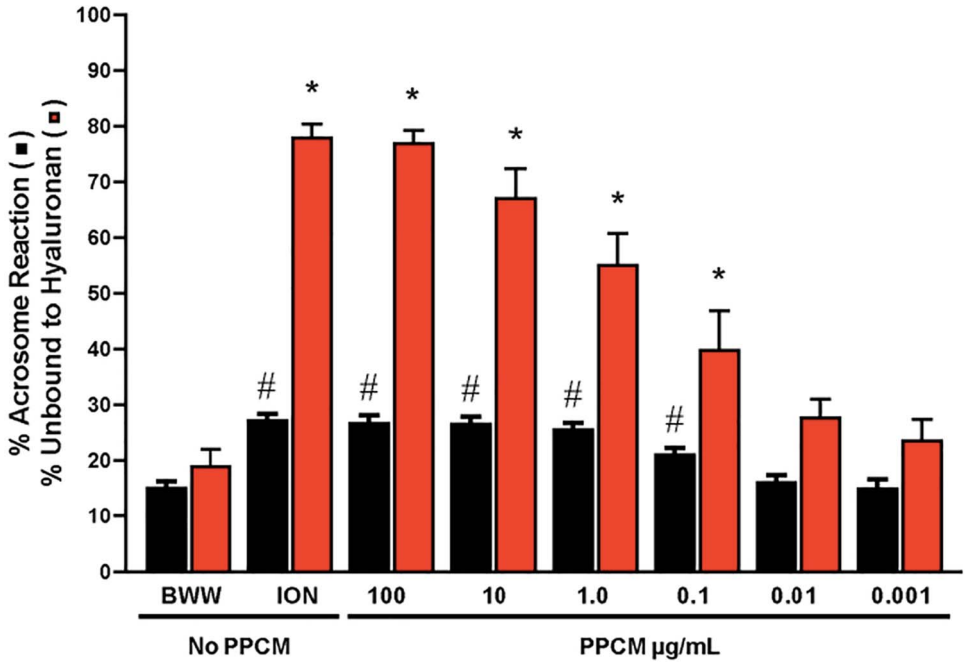


Figure 2. Comparison of sperm inactivation by PPCM measured by both an HBA and AS. Washed human sperm (20 million/mL) were resuspended in Biggers, Whitter, and Whittingham (BWW), divided into aliquots and incubated with increasing concentrations of PPCM for 15 min. Additional aliquots were incubated in BWW medium without PPCM (negative control) or with Ca++ ionophore A23187 (positive control). Mature, intact sperm bind to hyaluronan (HBA). The "% Unbound to Hyaluronan" represents the fraction of sperm inactivated by PPCM and unable to bind. Mature, intact sperm have an intact acrosome, which is identified by AS. Thus, "% Acrosome Reaction" represents the inactivated sperm with premature acrosome caused by PPCM. Both assays demonstrate dose-dependent increase in sperm inactivation (contraceptive activity) after exposure to PPCM. However, the HBA shows a greater effect and accounts for an 80% reduction, whereas AR shows maximal reduction of only 27%. Bars represent the mean ± SEM, n = 4–6/treatment group. #$P < 0.01$ PPCM at 100, 10, 1.0, and 0.1 vs. no PPCM in the HBA assay. *$P < 0.01$ PPCM at 100, 10, 1.0, and 0.1 ug/mL vs. no PPCM in the acrosome reaction assay.

given the millions of women who would benefit from these new products.

Acknowledgements

We thank G. Prins, W. Birch, L. Birch, K. Feathergill, and C. DeJonge for participating in the evaluation of the HBA biomarker.

Conflict of Interest

The authors have declared that no conflict of interest exists.

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