

GM-144, a Novel Lipophilic Vaginal Contraceptive Gel-Microemulsion

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ABSTRACT In a systematic effort to develop a dual-function intravaginal spermicide as well as a drug delivery vehicle against sexually transmitted pathogens, a submicron particle size (30-80 nm), lipophilic and spermicidal gel-microemulsion (viz GM-144) containing the pharmaceutical excipients propylene glycol, Captex 300, Cremophor EL, Phospholipon 90G, Rhodigel, Pluronic F-68, and sodium benzoate was formulated. GM-144 completely immobilized sperm in human or rabbit semen in less than 30 seconds. Therefore, the *in vivo* contraceptive potency of intravaginally applied GM-144 was compared in the standard rabbit model to those of the detergent spermicide, nonoxynol-9 (N-9)-containing formulation. Eighty-four ovulated New Zealand White rabbits in subgroups of 28 were artificially inseminated with and without intravaginal administration of GM-144 or 2% N-9 (Gynol II) formulation and allowed to complete term pregnancy. GM-144 showed remarkable contraceptive activity in the rigorous rabbit model. When compared with control, intravaginal administration of GM-144 and Gynol II resulted in 75% and 70.8% inhibition of fertility ($P < .0001$ versus control, Fisher's exact test), respectively. Thus, GM-144 as a vaginal contraceptive was as effective as the commercially available N-9 gel. In the rabbit vaginal irritation test, none of the 6 rabbits given daily intravaginal application of spermicidal GM-144 for 10 days developed epithelial ulceration, edema, leukocyte influx, or vascular congestion characteristic of inflammation (total score = 5). Therefore, GM-144

has the potential to become a clinically useful safe vaginal contraceptive and a vehicle for formulating lipophilic drugs used in reducing the risk of heterosexual transmission of sexually transmitted diseases.

KEYWORDS: Contraceptives, Gel-microemulsion, Intravaginal, Microbicide, Nonoxynol-9, Spermicide

INTRODUCTION

Presently available spermicidal contraceptives typically comprise detergent ingredients that disrupt cell membranes, including the neutral surfactants isononyl-phenyl-polyoxyethylene (9) ether or nonoxynol-9 (N-9), *p*-menthanyl-phenyl-polyoxyethylene (8,8) ether or menfegol, and isooctyl-phenyl-polyoxyethylene (9) ether or octoxynol-9 (O-9) [1, 2]. N-9 is the most commonly used spermicidal contraceptive in the United Kingdom and the United States [3, 4]. Worldwide, the cationic surfactant benzalkonium chloride and the anionic detergent sodium docusate (dioctyl sodium sulfosuccinate) are also used as vaginal spermicides [5]. Detergent-type spermicides have been used for more than 30 years in creams, gels, foams, and sponges, suppositories, and more recently in film and condom lubricants. N-9 is being used at concentrations of 2% to 6% in creams and gels, 12% in foams, and as high as 18% in condom lubricants and 28% in vaginal contraceptive film.

The spermicidal activities of detergent-type contraceptives are associated with their structural affinity to the membrane lipids [6, 7]; therefore, the major drawback of using N-9 or other currently used surfactants is their detergent-type effect on epithelial

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cells and normal vaginal flora [8-12]. N-9 displays spermicidal and antibacterial/antiviral activity *in vitro* against pathogens responsible for sexually transmitted diseases (STDs) only at cytotoxic doses [13]. Frequent use of N-9 as a vaginal contraceptive/microbicide has been associated with an increased risk of vaginal or cervical infection, irritation, or ulceration [14-17]. In addition, detergent-type spermicides alter vaginal bacteria or flora, and lead to an increased risk of opportunistic infections [18-20]. Such opportunistic infections are known to enhance the susceptibility of the ectocervical epithelium and the endocervical mucosa to sexually transmitted pathogens including human immunodeficiency virus, type 1 (HIV-1) infection [21-22]. Since heterosexual transmission of HIV-1 is the predominant mode of the epidemic spread of acquired immunodeficiency syndrome (AIDS), new, effective, acceptable, and safe vaginal spermicides lacking detergent-type membrane toxicity may offer significant clinical advantage over the currently available detergent spermicides.

Because vaginal spermicides will likely be used for decades to come, an ideal spermicide should be nontoxic to genital tract epithelial cells, should be inexpensive and be produced from commonly available resources, and should have a broad specificity for solubilizing the drugs for prevention of sexual transmission of several STDs including HIV-1. Microemulsion-based formulations that offer rapid dispersion and enhanced drug absorption profile can be exploited for the development of dual-function contraceptives. Microemulsions are thermodynamically stable, isotropically clear dispersions of water, oil, and surfactants with potential as drug-delivery vehicles [23, 24]. Microemulsions appear to have the ability to deliver larger amounts of topically applied agents into the mucosa than do traditional lotions and creams because they provide a better reservoir for a poorly soluble drug through their capacity for enhanced solubilization. A drug that is dissolved rather than suspended in a vehicle is in a form immediately available for absorption and is therefore generally more rapidly and more effectively absorbed [24].

In a systematic effort to develop a spermicidal gel-microemulsion with good solubility for lipophilic drugs, several components of microemulsion formulations were evaluated for their spermicidal property, drug solubility, particle size, stability, and responses to *in vivo* and *in vitro* biological models. A novel submicron (30-80 nm) particle gel-microemulsion (GM) formulation (viz GM-144), prepared from 7 nontoxic pharmaceutical excipients commonly used in topical, oral, and injectable medications, was found to have rapid sperm-immobilizing activity in human semen. In the standard rabbit model, GM-144, when tested as a vaginal contraceptive, was as effective as the commercially available N-9 gel formulation (Gynol II). No toxic effect on the vaginal mucosa of rabbits was observed after daily exposure for 10 days. Therefore, GM-144 has the potential to become a clinically useful safe vaginal contraceptive and as a vehicle for formulating lipophilic drugs used in reducing the risk of heterosexual transmission of STDs.

MATERIALS AND METHODS

Materials

Propylene glycol was obtained from Spectrum Quality Products Inc, New Brunswick, NJ. Captex 300 was from ABITEC Corp, Janesville, WI. Cremophor EL was purchased from BASF Corp, Mount Olive, NJ. Phospholipon 90G was purchased from American Lecithin Co, Danbury, CT. Rhodigel was from R.T. Vanderbilt Co, Norwalk, CT; Pluronic F-68 was obtained from JRH Biosciences, Inc, Lenexa, KS. N-9 (IGEPAL CO-630) was a generous gift from Rhone Poulenc, Cranbury, NJ.

GM-144 Formulations

A lipophilic submicron (30-80 nm) particle size microemulsion, GM-144, was developed using commonly used pharmaceutical excipients through systemic mapping of ternary-phase diagrams [23, 24]. The components of GM-144 formulation are listed in **Table 1**. The ingredients selected included drug solubilizers and stabilizers (propylene glycol, Captex 300, Cremophor EL, Phospholipon 90G, Pluronic F-68), and a preservative (sodium benzoate). A polymer

suspension of xanthan gum (Rhodigel) was selected as an additive to the microemulsion-based system to obtain a gel with desirable viscosity (1000 centipoise) with high thickening capability and compatibility with vaginal mucosa. The polymer did not cause precipitation or alter the microemulsion particle size. The GM-144 was found to be stable at ambient temperature. Particle size was determined using Nicomp Model 380 laser diode source (Particle Sizing Systems, Santa Barbara, CA). Viscosity measurements were made using the Brookfield Digital Viscometer (Model DV-II+; Brookfield Engineering Laboratories, Spoughton, MA).

Table 1. Components of GM-144 formulation

Ingredient	Type	Final concentration (% by weight)
Propylene glycol	Humectant	17.0
Captex 300	Lipid	4.5
Cremophor EL	Surfactant	3.8
Phospholipon 90G	Phospholipid	3.0
Rhodigel	Natural polymer	1.0
Pluronic F-68	Surfactant	0.4
Sodium benzoate	Preservative	0.2
Water	Diluent	70.1

Computer-Assisted Spermicidal Assay

All donor semen specimens were obtained after informed consent and in compliance with the guidelines of the Parker Hughes Institute Institutional Review Board. The kinetics of spermicidal activity of individual components of GM-144 were quantitated using a computer-assisted sperm analyzer (Hamilton Thorne Research [Danvers, MA] Integrated Visual Optical System, version 10.9i instrument) [8, 10, 11]. The ingredients evaluated were propylene glycol (17.0%), Captex 300 (4.5%), Cremophor EL (3.8%), Phospholipon 90G (3.0%), Rhodigel (1.0%), Pluronic F-68 (0.4%), and sodium benzoate (0.2%). The effect of incubation duration on sperm-head, centroid-derived sperm motility parameters was tested by mixing an aliquot of semen with an equal volume of each of the 7 pharmaceutical excipients or GM-144 formulation in Biggers, Whitten, and Whittingam’s medium (BWW) containing 25 mM HEPES (Irvine Scientific, Santa Ana, CA) and 0.3% BSA (BWW-

0.3% BSA) to yield the final concentrations contained in GM-144. At timed intervals of 1, 15, 30, 45 and 60 minutes, 5-mL samples were transferred to 2 20-mm Microcell (Conception Technologies) chambers; sperm motility was assessed by computer-assisted sperm analysis (CASA). Sperm motility in viscous samples (Phospholipon 90G and Rhodigel) was determined by manual phase-contrast microscopy (Olympus BX40; Olympus Corporation, Lake Success, NY), and the number of motile sperm per treatment were enumerated for 200 sperm. The time course test was performed in 3 separate trials, with semen obtained from 5 different donors.

Sperm Kinematic Parameters

For CASA, 5-µLs each of sperm suspension was loaded into 2, 20-µm Microcell chambers placed onto a counting chamber at 37°C; 5 to 8 fields per chamber were scanned for analysis. Each field was recorded for 30 seconds. The Hamilton Thorne computer calibrations were set at 30 frames at a frame rate of 30 images/second. Other settings were as follows: minimum contrast, 8; minimum size, 6; low-size gate, 1.0; high-size gate, 2.9; low-intensity gate, 0.6; high-intensity gate, 1.4; phase-contrast illumination; low path velocity, 10 µm/sec and threshold straightness, 80%; and magnification factor, 1.95. The performance of the analyzer was periodically checked using the playback function.

The sperm kinematic parameters evaluated included numbers of motile (MOT) and progressively (PRG) motile sperm; curvilinear velocity (VCL); average path velocity (VAP); straight-line velocity (VSL); beat-cross frequency (BCF); and the amplitude of lateral head displacement (ALH) and the derivatives, straightness ($STR = VSL/VAP \times 100$) and linearity ($LIN = VSL/VCL \times 100$). Data from each individual cell track were recorded and analyzed. For each aliquot sampled, more than 200 sperm were analyzed. The percentage motilities were compared with those of sham-treated control suspensions of motile sperm. The spermicidal activity of the test compound was expressed as $t_{1/2}$ values (the time taken to decrease the proportion of motile sperm by 50%).

Modified Sander-Cramer Assay

The spermicidal activity of GM-144 formulation with and without Rhodigel as well as the oleaginous nonisotropic mixture of GM-144 components was tested by a modified Sander-Cramer assay [8, 25]. Briefly, aliquots (0.1 μ Ls) of freshly liquefied semen were rapidly mixed with an equal volume of freshly prepared GM-144 formulation. A 5- μ L sample was transferred to a 20 μ m Microcell chamber (Conception Technologies) and examined immediately under a phase contrast microscope attached to a charge coupled device (CCD) camera (Hitachi Deneshi Ltd, Tokyo, Japan) and a video monitor. A commercial 2% N-9 formulation (Gynol II; Ortho Pharmaceutical Corp, Raritan, NJ) was used as a positive control. The time required for sperm immobilization was recorded in seconds. This test was performed in 9 separate trials, with fresh semen obtained from 5 different donors.

To assess the effect of decreasing concentrations of GM-144 and Gynol II on sperm immobilization, aliquots of liquefied semen (1:1) were mixed with serial 2-fold dilutions (50%-0.78%) of GM-144 or Gynol II in phosphate-buffered saline. We then recorded the dilution that induced more than 90% sperm immobilization following a 2-minute incubation.

Rabbits

Ninety female and 12 male, sexually mature (> 6 months old; > 4 kg), specific-pathogen-free, New Zealand White rabbits were obtained from Charles River Laboratories (Wilmington, DE). For each fertility trial, 14 does and 12 bucks were used. All rabbits were identified with specific metal ear tags. Tap water and rabbit food pellets (Teklad Hi-Fiber Diet #7012; Harlan Teklad, Madison, WI) were available ad libitum. The does and bucks were maintained in separate rooms that were kept at 22°C \pm 2°C with relative humidity of 50% \pm 20% and a 12-hour light:dark cycle. The rabbits were isolated for a minimum of 4 weeks before the fertility trials. All procedures were approved by the Parker Hughes Institute Institutional Animal Use and Care Committee. All animal husbandry operations were conducted under current US Department of Agriculture Guidelines.

In Vivo Contraceptive Efficacy in the Rabbit Model

For each contraceptive test, the does were divided into 3 subgroups of 14: control does, GM-144 group, and N-9 group. Semen was obtained from bucks (n = 12) of proven fertility via a prewarmed (45°C) artificial vagina immediately before use. Sperm count and motility were assessed to ensure that the males were ejaculating good-quality semen. Before artificial insemination, semen samples without the contamination of urine or gel were pooled and 0.5 mL (> 30 X 10⁷ sperm/mL) aliquots were transferred to 1 mL tuberculin syringes. Two mL of a GM-144 formulation (1000 centipoise) or Gynol II (120 000 centipoise) was applied intravaginally via a plastic tubing to a depth of 8 cm. The doe was held in a supine position during the application of 2 mL of the test agent followed by the application of 0.5 mL semen dose, which was deposited within 1 minute by inserting the tuberculin syringe into the vagina to a depth of 6 cm. At the time of artificial insemination, ovulation was induced by an intravenous injection of 100 IU of human chorionic gonadotropin (hCG) (Sigma Chemical Co, St Louis, MO) into the marginal ear vein. After ovulation and artificial insemination, the does were allowed to complete their pregnancy (31 \pm 2 days). Pregnant does were transferred to cages containing nest boxes (16 X 12 X 6 inches). The litter size and the weight and condition of each offspring at birth were recorded. The *in vivo* spermicidal effect of GM-144 formulation versus 2% N-9 formulation was assessed based on the level of pregnancy reduction achieved in comparison to controls and the consistency of this response. The vaginal delivery/artificial insemination and pregnancy cycle was repeated a second time.

Vaginal Irritation Test in Rabbits

For the vaginal irritation study, 6 female rabbits were treated intravaginally with 1 mL of GM-144 formulation, once per day for 10 consecutive days. Animals were killed on day 11 and the reproductive tract was examined grossly and microscopically [26]. The vaginal tissues were rapidly removed and parts of the upper (cervico-vagina), middle, and lower (uro-vagina) regions of each vagina were fixed in 10%

neutral-buffered formalin. Tissues were embedded in paraffin, sectioned at 4 to 6 mm and stained with hematoxylin and eosin and examined under X200 and X400 magnification using a Leica light microscope (Milton Keynes, Buckinghamshire, United Kingdom) interfaced with an image analysis system (Media Cybernetics, Silver Spring, MD) in conjunction with a 3-CCD camera (DAGE-MTI Inc, Michigan City, IN) for observation and analysis. Each of the 3 regions of vagina was examined for epithelial ulceration, edema, leukocyte infiltration, and vascular congestion. The irritation scores were assigned based on the scoring system of Eckstein et al, [26], which classifies levels of vaginal irritation as follows. Individual score: 0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = intense. The total scoring system correlates to human irritation potential as follows: total scores of 0 to 8 are acceptable, scores of 9 to 11 indicate borderline irritation potential, and scores greater than 12 are potentially irritating. Results were expressed as the mean \pm standard deviation values.

Statistical Analysis

Nonlinear regression analysis was used to find the $t_{1/2}$ values from the time-dependent motility loss curves using Graphpad Prism (version 3.0) software (San Diego, CA). The statistical significance of differences in fertility among the groups was analyzed by Fisher's exact test. Differences were considered statistically significant if $P < .05$.

RESULTS

Rapid Spermicidal Activity of GM-144 in Human Semen

The effect of individual components of GM-144 on the motility of sperm in human semen was evaluated by CASA. As shown in **Figure 1**, at the final concentrations used for GM-144 formulation, Captex 300, Cremophor EL, Phospholipon 90G, Pluronic F-68, and sodium benzoate demonstrated little or no inhibitory effects on human sperm motility ($t_{1/2} = > 60$ min). Further, sperm motion kinematics using CASA confirmed that these excipients did not significantly alter the sperm

motion parameters, such as the progressive velocity, straightness of the swimming pattern, linearity of the sperm tracks, beat-cross frequency, and the amplitude of lateral sperm head displacement (data not shown), whereas treatment of human semen with propylene glycol and Rhodigel at the concentration used for GM-144 formulation induced only partial spermicidal activity with slow kinetics ($t_{1/2} = > 24$ min). Progressive sperm motility ($> 30\%$) was evident even after 60 minutes of exposure to these components. Similarly, the microemulsion without the Rhodigel polymer was partially spermicidal with slow kinetics ($t_{1/2} = > 15$ min). The oleaginous nonisotropic mixture of microemulsion components lacked spermicidal activity in semen. By contrast, the submicron particle size gel-microemulsion (GM-144) containing Rhodigel as the polymer completely immobilized sperm in human semen in less than 30 seconds (mean 27 ± 4 sec; $n = 9$) in the modified Sander-Cramer assay. Even a 1:16 dilution of GM-144 induced more than 50% inhibition of sperm motility in semen after a 2-minute exposure. Thus, the combination of commonly used pharmaceutical excipients as a gel-microemulsion formulation was highly spermicidal in human semen.

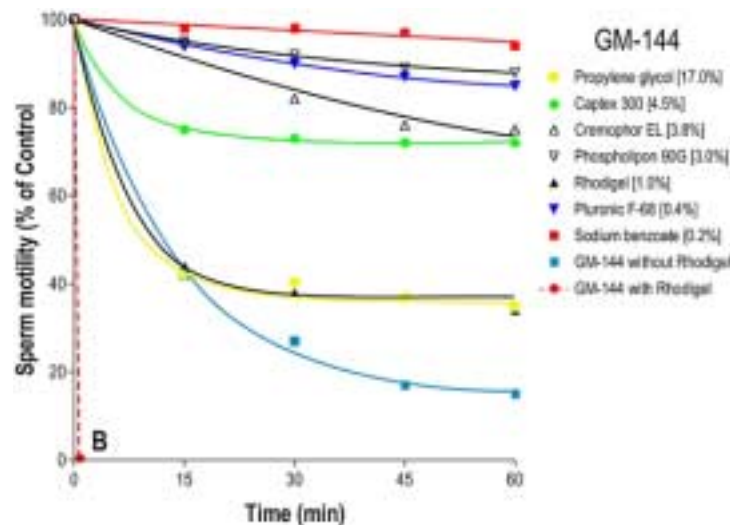


Figure 1. Effect of GM-144 and individual components of GM-144 on the motility of human sperm in semen.

***In Vivo* Contraceptive Activity of GM-144 versus N-9 Formulation in the Rabbit Model**

Because of the rapid spermicidal activity of GM-144, *in vivo* contraceptive efficacy studies of GM-144 in the standard rabbit model were performed. Gynol II, a commercial contraceptive gel containing 2% N-9, was tested in the same way for comparison. In the modified Sander-Cramer assay, Gynol II completely immobilized all sperm in human or rabbit semen in less than 20 seconds (mean 13 ± 2 seconds). A 1:32 dilution of Gynol II induced > 90% inhibition of sperm motility in semen after a 2-minute exposure and > 60% of sperm were immobilized at a 1:128 dilution.

For *in vivo* contraceptive efficacy studies, 84 ovulated New Zealand White rabbits in subgroups of 28 were artificially inseminated with fresh, pooled semen with and without intravaginal application of GM-144 formulation or N-9 and allowed to complete term pregnancy. The efficacy of GM-144 formulation versus Gynol II for preventing pregnancy in the rabbit model is summarized in **Table 2**. In the control group, 24 of 28 (85.7%) rabbits artificially inseminated became pregnant and delivered a total of 185 newborn rabbits. By contrast, only 6 of 28 (21.4%) rabbits given GM-144 formulation before artificial insemination became pregnant ($P < .0001$, Fisher's exact test) with a total of 34 newborn pups. Similarly, only 7 of 28 (25%) rabbits given Gynol II became pregnant ($P < .0001$, Fisher's exact test) and delivered a total of 47 newborn rabbits. Thus, the GM-144 formulation was as effective as Gynol II as a vaginal spermicidal contraceptive. Rabbits that delivered litters following single intravaginal application of GM-144 or Gynol II before artificial insemination had healthy offsprings with no perinatal or postnatal repercussions.

Lack of Vaginal Irritation from GM-144 in the Rabbit Model

Histological evaluation of 3 different regions of the vaginal tissues of 6 rabbits given daily intravaginal application of GM-144 for 10 consecutive days showed lack of significant vaginal irritation (mean individual scores 0-2; total score 5) (**Table 3**). None of the 6 rabbits treated with GM-144 revealed epithelial ulceration, edema, leukocyte influx, and

vascular congestion characteristic of inflammation as quantitated by histological scoring according to the method of Eckstein et al [26].

Table 2. Fertility of Female Rabbits after Artificial Insemination/Ovulation Induction with and without Intravaginal Application of GM-144 Formulation or Gynol II Containing 2% N-9

Treatment*	No. of does inseminated	No. of does fertile (%)	Mean litter size (median)	Total litter size
None	28	24 (85.7%)	7.7 ± 3.3	185
GM-144	28	6 (21.4%) [†]	5.6 ± 3.1	34
Gynol II (2% N-9)	28	7 (25.0%) [†]	6.7 ± 1.0	47

*Aliquots (0.5 mL) of fresh, pooled semen obtained from fertile bucks (n = 12) were used to artificially inseminate the does within 1 minute after intravaginal application of 2 mL of GM-144 formulation or Gynol II (2% N-9 gel). Does were induced to ovulate by an intravenous injection of 100 IU of hCG and allowed to complete term pregnancy.

[†]Significantly different from control by Fisher's exact test ($P < .0001$).

Table 3. Scoring of Histological Changes in the Rabbit Vaginal Tissue after 10 Days of Intravaginal Application of GM-144 Formulation

	Cervico-vagina (n = 6)	Mid-vagina (n = 6)	Uro-vagina (n = 6)
Epithelial ulceration	0*	0	0
Lamina propria thickness	1 ± 1 ^{†,‡}	1 ± 1	1 ± 1
Leukocyte infiltration	2 ± 1	2 ± 1	2 ± 1
Vascular congestion	2 ± 1	2 ± 1	2 ± 1
Total score	5 ± 1	5 ± 1	5 ± 1

*Six rabbits were administered 1 mL of GM-144 daily for 10 days intravaginally.

[†]Mean \pm SD values representing the upper (cervico-vagina), middle, and lower (uro-vagina) regions of vagina from 6 rabbits.

[‡]Semiquantitative scoring criterion adapted from Eckstein et al [26]. Individual score: 0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = intense. Correlation to human irritation potential: total score < 8 acceptable, 9-10 marginal, and >11 unacceptable.

DISCUSSION

The individual components of GM-144 formulation as well as its oleaginous nonisotropic mixture lacked rapid spermicidal activity in semen, whereas the gel-microemulsion, GM-144, containing all 7 pharmaceutical excipients rapidly inactivated sperm in human semen. The kinetics of the *in vitro* spermicidal activity of oil/water microemulsion in semen was dramatically enhanced by the addition of the gel polymer, Rhodigel, clearly demonstrating the synergistic effect of gel-microemulsion. The rapid spermicidal property of the lipophilic gel-microemulsion, GM-144, has potential to provide improved methods of vaginal contraceptives in addition to being a drug-delivery vehicle for formulating lipophilic drugs used in reducing the risk of heterosexual transmission of STDs.

Since the rabbit provides a standard animal model for testing vaginal agents for antifertility activity [27], the ability of intravaginally applied GM-144 to prevent pregnancy in ovulated rabbits was tested. Vaginal delivery of GM-144 formulation before artificial insemination was found to drastically reduce pregnancy rates in the rigorous rabbit model through *in vivo* contraceptive efficacy studies which included term pregnancy as well as the analysis of normalcy of the resulting pregnancies. Intravaginal application of GM-144 before artificial insemination resulted in a 75% contraceptive effect despite the fact that the rabbit ejaculate used in our fertility trials was in the order of several hundred human ejaculates [28]. Under identical conditions, Gynol II showed 71% inhibition of fertility. In addition to the spermicidal property, the potent contraceptive effect of GM-144 may also be in part because of the known ability of microemulsions to alter membrane ionization potentials or oxidation-reduction properties, which are important for sperm-egg interactions.

Despite the rapid *in vitro* spermicidal property of N-9, the *in vivo* contraceptive activity of 2% N-9 in the rabbit model has been shown to be highly dependent on the time interval between delivering the agent to the vagina and artificial insemination or coitus [27]. In addition, a large excess of N-9 is required in the vagina to achieve effective *in vivo* contraception as

compared to the dose of N-9 needed to kill all sperm *in vitro* [27]. In the present study, the rabbit ejaculate used to inseminate the does was more than 1000-fold larger than that of humans [28]. Therefore, under the experimental conditions used, a partial contraceptive activity in rabbits observed with GM-144 and Gynol II, respectively, can be considered essentially 100% contraceptive in humans [27, 28].

The second objective of these studies was to determine the toxic effects, if any, resulting from repeated intravaginal application of spermicidal GM-144. Because of the potent *in vitro* and *in vivo* spermicidal activity of GM-144 formulation, it was necessary to evaluate the toxicity to vaginal mucosa particularly in the rabbit vaginal irritation test. A correlation exists between rabbits and humans with respect to the irritation potential of vaginal contraceptive compositions. Because the constituents of GM-144 are nontoxic drug solubilizers and polymers in the rabbit vaginal tolerance test, GM-144 lacked mucosal toxicity after daily application for 10 days. The histopathological evaluation clearly demonstrated that the GM-144 is not damaging to vaginal mucosa of the rabbit despite it being a potent spermicidal agent when added to human or rabbit semen. Because the spermicidal activity of GM-144 is not the result of a detergent-type mechanism, when used as a contraceptive it is unlikely to significantly affect or inhibit the growth characteristics of vaginal flora. Thus, the data suggest that unlike the currently used nonionic and cationic detergent spermicides, the submicron particle-based GM-144 formulation is not likely to cause harmful side effects after repetitive intravaginal application. Experiments to formally test the safety of the intravaginally applied spermicidal gel-microemulsion on the long-term health and reproductive performance of test animal species are currently in progress.

The components used for GM-144 formulation are nontoxic solubilizers for lipophilic drugs used in the preparation of a variety of topical, oral, and injectable medications. Propylene glycol (propane-1,2-diol), Captex 300 (medium chain triglyceride), Cremophor EL (polyethoxylated castor oil), Phospholipon 90G (purified soya lecithin), and Pluronic F-68 (poly[oxyethylene]-poly[oxypropylene]) are widely

used parenteral vehicles as nontoxic solubilizers for lipophilic drugs and vitamins [29-32]. Cremophor EL, when used up to 10% wt/vol, did not cause any apparent membrane damage to cell monolayers and did not cause lysis of human leukemic cells [33]. Pluronic F-68 is a nonionic polyol that does not have any intrinsic antibacterial activity. It is commonly used to protect cultured animal cells from the detrimental effects of sparging [34]. Pluronic poloxamers are being used to enhance absorption of drugs through the mucus membranes. Long-term toxicity studies and clinical trials suggest that these pharmaceutical excipients are safe for human use [35]. Rhodigel (xanthan gum) was preferred as a gel base because of its safety and wide acceptability as a pharmacological excipient for topical application [36].

The microemulsion-based lipophilic and vaginal spermicide, GM-144, appears to offer several benefits for vaginal delivery, including increased absorption, potent contraceptive activity, and decreased toxicity. As a potent contraceptive agent, which is inexpensive and devoid of mucosal toxicity, the lipophilic GM-144 formulation meets the criteria for a vaginal spermicide and warrants further preclinical evaluation. In addition, this nontoxic lipophilic gel-microemulsion may also be useful for intravaginal application of antimicrobial agents to prevent the sexual transmission of diseases such as AIDS, genital herpes, gonorrhea, and chlamydia.

CONCLUSIONS

A novel, lipophilic, submicron (30-80 nm)-particle-size gel-microemulsion, GM-144, prepared from pharmaceutical excipients commonly used in topical, oral, and injectable medications, was found to exhibit potent spermicidal activity, although these excipients by themselves exhibit little or no spermicidal activity in human semen. In the rabbit model, GM-144 as a vaginal contraceptive was as effective as the commercially available detergent-type spermicide, nonoxynol-9 (N-9) gel. Repeated intravaginal application of GM-144 in the rabbit vaginal irritation test was not associated with local inflammation or damage of the vaginal mucosa or epithelium. Therefore, GM-144 shows potential to become a clinically useful safe vaginal contraceptive and a

potential drug-delivery vehicle for formulating lipophilic drugs used in reducing the risk of heterosexual transmission of STDs.

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REFERENCES

1. Digenis GA, Nosek D, Mohammadi F, Darwazeh NB, Anwar HS, Zavos PM. Novel vaginal controlled-delivery systems incorporating coprecipitates of nonoxynol-9. *Pharm Dev Technol.* 1999;4:421-430.
2. Furuse K, Ishizeki C, Iwahara S. Studies on spermicidal activity of surfactants. I. Correlation between spermicidal effect and physicochemical properties of p-methanylphenyl polyoxyethylene (8.8) ether and other surfactants. *J Pharmacobiodyn.* 1983;6:359-372.
3. OTC Panel. Vaginal contraceptive drug products for over-the-counter human use. *Federal Register.* 1980;45:82014-82019.
4. Chantler E. Vaginal spermicides: some current concerns. *Brit Fam Plann.* 1992;17:118-119.
5. Mendez F, Castro A, Ortega A. Use effectiveness of a spermicidal suppository containing benzalkonium chloride. *Contraception.* 1986;34:353-362.
6. Schill WB, Wolf HH. Ultrastructure of human spermatozoa in the presence of the spermicide nonoxynol-9 and a vaginal contraceptive containing nonoxynol-9. *Andrologia.* 1981;13:42-49.
7. Wilburn WH, Hahn DW, McGuire JJ. Scanning electron microscopy of human spermatozoa after incubation with the spermicide nonoxynol-9. *Fertil Steril.* 1983;39:717-719.
8. D'Cruz OJ, Shih M-J, Yiv SH, Chen C-L, Uckun FM. Synthesis, characterization and preclinical formulation of a dual-action phenyl phosphate derivative of bromo-methoxy zidovudine (compound WHI-07) with potent anti-HIV and spermicidal activities. *Mol Hum Reprod.* 1999;5:421-432.

9. D'Cruz OJ, Venkatachalam TK, Uckun FM. Structural requirements for potent human spermicidal activity of dual-function aryl phosphate derivative of bromo-methoxy zidovudine (compound WHI-07). *Biol Reprod.* 2000;62:37-44.
10. D'Cruz OJ, Uckun FM. Novel derivatives of phenethyl-5-bromopyridylthiourea (PBT) and dihydroalkoxybenzylloxypyrimidine (DABO) are dual-function spermicides with potent anti-HIV activity. *Biol Reprod.* 1999;60:1419-1428.
11. D'Cruz OJ, Venkatachalam TK, Uckun FM. Novel thiourea compounds as dual-function microbicides. *Biol Reprod.* 2000;63:196-205.
12. Klebanoff SJ. Effects of the spermicidal agent nonoxynol-9 on vaginal microbial flora. *J Infect Dis.* 1992;165:19-25.
13. Uckun FM, D'Cruz OJ. Prophylactic contraceptives for HIV/AIDS. *Hum Reprod Update.* 1999;5:506-514.
14. Niruthisard SR, Roddy E, Chutivongse S. The effects of frequent nonoxynol-9 use on the vaginal and cervical mucosa. *Sex Transm Dis.* 1991;18:176-179.
15. Rekart ML. The toxicity and local effects of the spermicide nonoxynol-9. *J Acquir Immune Defic Syndr.* 1992;5:425-427.
16. Roddy RE, Cordero M, Cordero C, Fortney JA. A dosing of nonoxynol-9 and genital irritation. *Int J STD HIV.* 1993;4:165-170.
17. Weir SS, Roddy RE, Zekeng L, Feldblum PJ. Nonoxynol-9 use, genital ulcers, and HIV infection in a cohort of sex workers. *Genitourin Med.* 1995;71:78-81.
18. Hooten TM, Hillier S, Johnson C, Roberts PL, Stamm WE. *Escherichia coli* bacteriuria and contraceptive method. *JAMA.* 1991;265:64-69.
19. Stafford MK, Ward H, Flanagan A, et al. A safety study of nonoxynol-9 as a vaginal microbicide: evidence of adverse effects. *J Acquir Immune Defic Syndr Hum Retrovirol.* 1998;17:327-331.
20. Rosenstein IJ, Stafford MK, Kitchen VS, Ward H, Weber JN, Taylor-Robinson D. Effect of normal vaginal flora of three intravaginal microbicidal agents potentially active against human immunodeficiency virus type 1. *J Infect Dis.* 1998;177:1386-1390.
21. Augenbraun MH, McCormack WM. Sexually transmitted diseases in HIV-infected persons. *Infect Dis Clin North Am.* 1994;8:439-448.
22. Kreiss J, Ngugi E, Holmes K, et al. Efficacy of nonoxynol-9 contraceptive sponge use in preventing heterosexual transmission of HIV in Nairobi prostitutes. *JAMA.* 1992;268:477-482.
23. Eccleston GM. Microemulsion. In: Swarbrick J, Boylan JC, eds. *Encyclopedia of Pharmaceutical Technology.* New York: Marcel Dekker, 1992:375-421.
24. Tenjaria S. Microemulsions: an overview and pharmaceutical applications. *Crit Rev Ther Drug Carrier Syst.* 1999;16:461-521.
25. D'Cruz OJ, Zhu Z, Yiv SH, Chen C-L, Waurzyniak B, Uckun FM. WHI-05, a novel bromo-methoxy substituted phenyl phosphate derivative of zidovudine, is a dual-action spermicide with potent anti-HIV activity. *Contraception.* 1999;59:319-331.
26. Eckstein P, Jackson MC, Millman N, Sobrero AJ. Comparison of vaginal tolerance tests of spermicidal preparations in rabbits and monkeys. *J Reprod Fertil.* 1969;20:85-93.
27. Castle PE, Hoen TE, Whaley KJ, Cone RA. Contraceptive testing of vaginal agents in rabbits. *Contraception.* 1998;58:51-60.
28. Castle PE, Whaley KJ, Hoen TE, Moench TR, Cone RA. Contraceptive effect of sperm-agglutinating monoclonal antibodies in rabbits. *Biol Reprod.* 1997;56:153-159.
29. Lundberg BB. A submicron lipid emulsion coated with amphipathic polyethylene glycol for parenteral administration of Paclitaxel (Taxol). *J Pharm Pharmacol.* 1997;49:16-21.
30. Woodcock DM, Jefferson S, Linsenmeyer ME, Crowther PJ, Chojnowski GM, William B, Bertoncello I. Reversal of multidrug resistance phenotype with Cremophor EL, a common vehicle for water-insoluble vitamins and drugs. *Cancer Res.* 1990;5:4199-4203.

31. Dreher F, Walde P, Luisi PL, Elsner P. Human skin irritation studies of a lecithin microemulsion gel and of lecithin liposomes. *Skin Pharmacol.* 1996;9:124-129.
32. Katz DH, Marcelletti JF, Khalil MH, Pope LE, Katz LR. Antiviral activity of 1-docosanol, an inhibitor of lipid-enveloped viruses including herpes simplex. *Proc Natl Acad Sci U S A.* 1991;88:10825-10829.
33. Nerurkar MM, Burton PS, Borchardt RT. The use of surfactants to enhance the permeability of peptides through Caco-2 cells by inhibition of an apically polarized efflux system. *Pharm Res.* 1996;13:528-534.
34. Murhammer DW, Goochee CF. Sparged animal cell bioreactors: mechanism of cell damage and Pluronic F-68 protection. *Biotechnol Prog.* 1990;6:391-397.
35. de Jong HJ. The safety of pharmaceutical excipients. *Therapie.* 1999;54:11-14.
36. Sutherland IW. Novel and established applications of microbial polysaccharides. *Trends Biotechnol.* 1998;16:41-46.