Conceival, a Novel Noncontraceptive Vaginal Vehicle for Lipophilic Microbicides

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Osmond J. D'Cruz,[1,2,3] Peter Samuel,[1,4] and Fatih M. Uckun[1,5]

[1]Drug Discovery Program, Parker Hughes Institute, St Paul, MN 55113
[2]Department of Reproductive Biology, Parker Hughes Institute, St Paul, MN 55113
[3]Paradigm Pharmaceuticals, LLC, St Paul, MN 55113
[4]Department of Pharmaceutical Sciences, Parker Hughes Institute, St Paul, MN 55113
[5]Department of Virology, Parker Hughes Institute, St Paul, MN 55113

ABSTRACT

The objective of this study was to develop a nontoxic and noncontraceptive vaginal drug delivery vehicle for lipophilic anti–human immunodeficiency virus (HIV) microbicides. Three representative poorly water-soluble novel broad-spectrum anti-HIV microbicides, PHI-113, PHI-346, and PHI-443, were evaluated in 11 different solvent systems. Based on their solubility profiles, a novel nonspermicidal self-emulsifying gel (viz Conceival) composed of pharmaceutical excipients, sorbitol, polyethylene glycol 400, polysorbate 80, microcrystalline cellulose, xanthan gum, and water was optimized. Conceival enhanced the solubility of these poorly water-soluble (<0.001 mg/mL) anti-HIV drugs by at least 150- to 270-fold. Conceival was evaluated in vivo in the New Zealand white rabbit model for the preservation of sperm function based on pregnancy outcome and the potential for vaginal irritation following single and multiple intravaginal applications, respectively. Conceival administered intravaginally immediately prior to artificial insemination with semen had no adverse effects on subsequent reproductive performance, neonatal survival, or pup development when compared with untreated control group. Histologic evaluation of vaginal tissues of rabbits exposed intravaginally to Conceival for 14 consecutive days revealed lack of epithelial, submucosal, and vascular changes at the gel application site (total irritation score <3 out of a possible 16). These findings indicate that Conceival has potential to become a clinically useful, safe noncontraceptive vaginal vehicle for lipophilic microbicides.

KEYWORDS: HIV/AIDS, intravaginal, lipophilic gel, noncontraceptive, microbicide

Corresponding Author: Osmond J. D'Cruz, Drug Discovery Program, Parker Hughes Institute, St Paul, MN 55113; Tel: (651) 628-9988; Fax: 651-628-9891; E-mail: odcruz@ih.org

INTRODUCTION

Heterosexual transmission of human immunodeficiency virus (HIV-1) accounts for nearly 90% of all HIV-1 infections in women.[1,2] Currently, an estimated 19.2 million women worldwide are infected with HIV-1, accounting for ~50% of the 40 million adults living with HIV/AIDS.[3] In the United States, the proportion of HIV acquisition attributable to heterosexual transmission in women has increased from 21% in 1986 to 42% in 2002.[4,5] Women are 4 to 16 times more likely to contract HIV from infected males than vice versa, and young women are especially vulnerable.[6] In addition to HIV, an estimated 45 million Americans older than 14 years of age suffer from genital herpes (1 in 5), and 20 million from human papilloma virus (HPV) (1 in 10), and more than half a million each have sexually transmitted hepatitis B virus infection (HBV).[7]

The emergence of HIV/AIDS as a disease spread through sexual intercourse, combined with growing public awareness about the problems associated with other viral sexually transmitted infections (STIs), has prompted the search for new, cost-effective, and safe vaginal microbicides for curbing transmucosal viral transmission.[8,9] Microbicides would provide protection by inactivating viruses or preventing viruses from replicating either in semen or the infected host cells that line the vaginal wall. Microbicides that are currently being investigated are directed mainly at preventing pregnancy as well as protection against STIs.[10-12] The availability of a nonspermicidal microbicide will be equally important for (1) sexually active women to allow pregnancy, while protecting both mother and fetus from viral STIs including HIV-1, and (2) as a prophylactic antiviral agent, especially for HIV-1 serodiscordant couples, to curb the transmission of HIV-1 via semen prior to assisted reproductive technology procedures.

Yet many of the current antimicrobial agents intended for vaginal microbicides are poorly soluble in aqueous medium.[13] Poor solubility may lead to poor dissolution kinetics, suboptimal bioavailability, ineffective therapy, and the need for higher dosing, thereby contributing to vaginal irritation. Because vaginal microbicides would likely be used repeatedly over decades, an ideal microbicide should have an established safety record and lack

genital epithelial as well as submucosal toxicity. Moreover, the drug delivery vehicle 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 STIs including HIV-1.

In a systematic effort to develop a noncontraceptive drug delivery vehicle with good solubility for lipophilic drugs, several pharmaceutical excipients were evaluated for drug solubility, stability, and responses to in vivo and in vitro biological models. Three representative novel anti-HIV microbicides, PHI-113/Stampidine [2,′3′-didehydro-3′-deoxythymidine 5′-(p-bromophenyl methoxyalaninyl phosphate], PHI-346 [N-[2-(1-cyclohexenyl)ethyl]-N′-[2-(5-bromopyridyl)]thiourea], and PHI-443 [N′-[2-(2-thiophene)ethyl]-N′-[2-(5-bromopyridyl)]thiourea] were used as model drugs. They have broad-spectrum in vivo anti-HIV activity, lack of systemic and mucosal toxicity following repeated oral or intravaginal administration, and display favorable in vivo pharmacokinetic profiles.[11,14–20] A novel nonspermicidal gel (viz Conceival) composed of pharmaceutical excipients, sorbitol, polyethylene glycol 400, polysorbate 80, microcrystalline cellulose, xanthan gum, and water was formulated incorporating these lipophilic drugs. These pharmaceutical excipients were selected because of their safety, physical characteristics, bioadhesive properties, versatile consistencies, and solubility, as well as improved bioavailability of target drugs.[21–24] Conceival greatly enhanced the solubility of poorly water-soluble anti-HIV microbicides. In the rabbit model, Conceival lacked mucosal toxicity following repeated intravaginal application and did not affect in vivo fertility and birth outcome when administered at the time of artificial insemination. Conceival has potential to become a clinically useful, safe noncontraceptive vaginal vehicle for formulating lipophilic drugs as prophylactic microbicides.

MATERIALS AND METHODS

Anti-HIV Drugs

The anti-HIV drugs, PHI-113/Stampidine [2,′3′-didehydro-3′-deoxythymidine 5′-(p-bromophenyl methoxyalaninyl phosphate], PHI-346 [N-[2-(1-cyclohexenyl)ethyl]-N′-[2-(5-bromopyridyl)]thiourea], and PHI-443 [N′-[2-(2-thiophene)ethyl]-N′-[2-(5-bromopyridyl)]thiourea], were synthesized and purified as detailed in our previous reports.[17,19,25]

Excipients and Reagents

The following excipients were used to test the solubility profile of 3 lipophilic anti-HIV microbicides: microcrystalline cellulose, Avicel (FMC Corp, Newark, DE); Captex (Abitec Corp, Janesville, WI); carboxy methyl cellulose and oleic acid (Spectrum Chemical Manufacturing Co, Gardena, CA); Cremophor EL (BASF Corp, Mount Olive, NJ); Gelucire 50/13, Labrafil M, and Labrasol (Gattefosse, Saint Prest, Cedex, France); hydroxy stearate polyethylene glycol 660 (Solutol HS; BASF); nonoxynol-9 (IGEPAL CO-630; Rhone Poulenc, Cranbury, NJ); polyethylene glycol (PEG; Union Carbide Chemical and Plastic Co Inc, Danbury, CT); Polysorbate 80 (Croda Inc, Parsippany, NY); Sorbitol (70% solution; Spectrum Chemical Manufacturing Co); sodium benzoate (Cultor Food Science, Ardsley, NY); Rhodigel 200 (R. T. Vanderbilt Co Inc, Norwalk, CT); Vitamin E and Vitamin E DL-TPGS (a-tocopheryl PEG 1000 succinate; Peebock Division of Eastman Co UK Ltd, Llangefni, Anglesey, UK); Xantural 75, 200 mesh (Pharmaceutical Ingredients, Norristown, PA); and deionized distilled water was purified via the Millipore Milli-Q purification system (Medford, MA).

For high-performance liquid chromatography (HPLC) analysis of anti-HIV drugs, the chemicals used were of analytical reagent grade. HPLC-grade acetonitrile was purchased from Burdick and Jackson (Muskegon, MI). All other chemicals were purchased from Aldrich (Milwaukee, WI), Sigma Co (St Louis, MO), or Fisher Scientific (Pittsburgh, PA) and used without further purification.

Partition Coefficients

The octanol/water partition coefficient was determined by the shake flask method. Representative anti-HIV drugs were mixed with equal volumes of water and octanol for 4 hours, and the 2 phases were separated, filtered, and analyzed by HPLC. The partition coefficient was calculated using the ratio of the area under the curve for octanol and water, respectively.

Gel Formulation

A lipophilic gel (viz, Conceival) capable of incorporating lipophilic drugs was formulated using commonly used pharmaceutical grade excipients through systematic drug solubilization studies.[13,26] The ingredients selected included drug solubilizers and stabilizers (PEG, polysorbate 80, and Sorbitol), and a preservative (sodium benzoate). A polymer suspension of microcrystalline cellulose (Avicel) and xanthan gum (Xantural 75) were selected as a thickening agent additive to the lipid-based system to obtain a gel with desirable viscosity and compatibility with vaginal mucosa. In brief, an admixture of microcrystalline cellulose and xanthan gum in water was prepared to obtain a viscous solution. Further addition of sorbitol to the solution resulted in the gel that was used as the suspending medium. To this gel-sorbitol formulation was added PEG solution of the drug followed by the surfactant polysorbate

80 to obtain the final gel formulation. The pH of the final gel formulation was 6.1. Viscosity measurements were made using the Brookfield digital viscometer (model DV-II+; Brookfield Engineering Laboratories, Stoughton, MA).

Dissolution Rate Studies

The dissolution rate of PHI-113 was studied in synthetic vaginal fluid (pH = 4.2) and in various dissolution media (pH range 2–12) using United States Pharmacopeia apparatus I method (basket, USP) on a Vankel 750 dissolution apparatus (USP, 2000).[27,28]Briefly, a 1% PHI-113 in Conceival (wt/wt) was loaded in size 00 hard gelatin capsules (Capsugel Corp, Greenwood, SC). The dissolution conditions were as follows: temperature 37°C ± 0.5°C, volume 500 mL, spindle speed 100 rpm. Three replicate runs were performed for each time point. Samples were withdrawn at 5, 15, 30, 60, 90, and 120 minutes and assayed using HPLC.[28] The dissolution profile was expressed as percentage drug released versus time.

HPLC Analysis

Chromatographic analysis of PHI-113, PHI-346, and PHI-443 were performed using a previously established and validated HPLC method.[29] The HPLC system used for these studies was a Hewlett Packard (Agilent Technologies, Palo Alto, CA) series 1100 instrument equipped with a quaternary pump, an auto sampler, an automatic electronic degasser, an automatic thermostatic column compartment, a diode array detector, and a computer with ChemStation software for data analysis. The analytical column used was a reverse phase Lichrospher 100, RP-18 column (5 μM; 4 × 250 mm) attached to a Lichrospher 100, RP-18 guard column (5 μM; 4 × 4 mm). An isocratic condition was employed for the mobile phase (vol/vol) with a flow rate of 1 mL/min. The composition of the mobile phase for each drug is as follows: PHI-113 (acetonitrile + 0.1% triethyl amine and 0.1% trifluoroacetic acid in water = 77:23); PHI-346 (acetonitrile + 0.1% triethyl amine and 0.1% trifluoroacetic acid in water = 77:23); and PHI 443 (acetonitrile + 0.1% acetic acid = 35:65). The UV detection wavelengths for PHI-113, PHI-346, and PHI-443 were at 265, 265, and 275 nm, respectively. Their corresponding retention times were 8.0, 7.8, and 9.1 minutes, respectively. The run time for the drugs was kept at 15 minutes.

Excipient Compatibility Studies

An accelerated excipient compatibility of PHI-113, PHI-346, and PHI-443 with formulation ingredients was performed. Briefly, the drugs were dissolved in ethanol and mixed with the formulation ingredients in the presence of water and a large excess of the excipients. Three replicates for each excipient at each temperature were prepared. These samples were stored at 50°C, and a control sample was stored in the freezer (−20°C). The samples were assayed by HPLC for the drug content after 24 hours.

For determining the amount of drug in each formulation, a known weight of Conceival was extracted with acetonitrile over a period of 4 hours. This solution was then used for quantitative determination of the respective drugs using HPLC methods described above. The concentration of the drug was calculated from an external calibration curve, generated for each drug, using a known concentration of the respective reference drug substance.

Assessment of Sperm Motility

Human sperm motility following exposure to various pharmaceutical grade excipients and their formulations was assessed using a modified Sander-Cramer assay.[13,30] Donor semen specimens were obtained after informed consent and in compliance with the guidelines of the Parker Hughes Institute Institutional Review Board. Briefly, aliquots (0.1 mL) of freshly liquefied human semen (n = 5) were rapidly mixed with an equal volume of Conceival. A 5-μL sample was transferred to a 20-μm Microcell chamber (Conception Technologies, San Diego, CA) and examined immediately under a phase contrast microscope attached to a charge coupled device 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. This test was performed in 12 separate trials, with fresh semen obtained from 5 different donors.

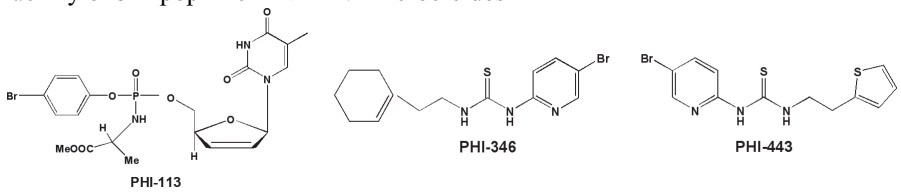
Animal Study

One hundred and seventeen sexually mature female and 48 male New Zealand White (NZW) rabbits were obtained from either Charles River Canada (St Constant, Quebec, Canada) or Harlan (Oakwood, MI). They were housed in separate rooms that were kept at 20°C ± 2°C with relative humidity of 50% ± 10% with a 12-hour fluorescent lighting cycle. Tap water and rabbit food pellets (Teklad 7015; Harlan Teklad, Madison, WI) were available ad libitum. Animal studies were approved by the Parker Hughes Institute Animal Use and Care Committee and all animal care procedures were conducted according to the current United States Department of Agriculture (USDA) Guidelines.

In Vivo Fertility Trials

Experiment 1 evaluated the effect of intravaginally administered Conceival prior to artificial insemination (AI) with semen on in vivo pregnancy rates. Pregnancy was deter-

Table 1. Comparative Solubility of 3 Lipophilic Anti-HIV Microbicides



Solvent	PHI-113 (mg/mL)	PHI-346 (mg/mL)	PHI-443 (mg/mL)
Water	3.0	0.001	0.001
10% Cremophor EL	10.4	0.52	0.24
10% Polysorbate 80	8.7	0.43	0.27
Polyethylene glycol (PEG-400)	>20	>10	28
Propylene glycol	>20	1.18	18.2

mined by counting the number of embryos 8 days after ovulation induction and AI. Thirty-eight does and 24 bucks were used. The does were divided into 2 subgroups of 20 and 18: untreated control and Conceival group, respectively. Semen was obtained from bucks of proven fertility via an artificial vagina (45°C) immediately before use.[31] Sperm count and motility were assessed by computerized sperm analysis (CASA; Hamilton Thorne Integrated Visual Optical System [IVOS], version 10.9i, Hamilton Research Inc, Danvers, MA). Semen samples without the contamination of urine or gel were pooled ($>10^8$ motile sperm/mL) and divided into 0.5-mL aliquots. Fifteen minutes prior to AI, 2 mL of Conceival was deposited intravaginally at a depth of 8 cm followed by deposition of 0.5 mL semen to a depth of 5 to 6 cm. Ovulation was induced at the time of AI by an intravenous injection of 100 IU of human chorionic gonadotropin (hCG) into the marginal ear vein. On postinsemination day 8, does were killed humanely, the uteri and the ovaries were excised, and the total numbers of embryos in each uterine horn and the number of corpora lutea in each ovary indicative of ovulated eggs were determined. The ratio of embryos to corpora lutea was used as a measure of fertility.

Experiment 2 evaluated the effect of intravaginally administered Conceival prior to AI with semen on term pregnancy and birth outcome. Sixty-seven does and 24 bucks were used. The does were divided into 3 subgroups of 20, 22, and 25 as follows: (1) untreated control does, (2) does treated with 1 mL of Conceival, and (3) does treated with 2 mL of Conceival. One or 2 mL of Conceival was applied intravaginally to hormonally primed does, which were then artificially inseminated with 0.5 mL semen as described above and allowed to complete their pregnancies (31 ± 2 days). The litter size as well as the weight and condition of each offspring at birth were recorded. Pregnancy rates were calculated as the proportion of does that became pregnant and delivered newborn rabbits. The pups were allowed to remain with the dam until day 5. The number of pups per litter and the mean weight of the pups surviving on day 5 were used to measure the perinatal effects of Conceival.

Rabbit Vaginal Irritation Test

For the vaginal irritation study, 9 female rabbits in 3 replicates of 2, 3, or 4 rabbits/treatment group were administered intravaginally with 1 mL of Conceival for 14 consecutive days. As a positive control, 3 rabbits were administered 1 mL gel containing 4% N-9. Animals were humanely killed on day 15, and parts of the cervico-vagina, mid-vagina, and uro-vagina of each animal were fixed in 10% neutral-buffered formalin. Fixed vaginal tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Stained sections were examined by light microscopy by a board-certified veterinary pathologist. Each of the 3 regions of vagina was scored blindly for epithelial ulceration, leukocyte infiltration, edema, and vascular congestion. The irritation scores were assigned based on the semiquantitative scoring system of Eckstein et al,[32] which was as follows: individual score, 0 = none; 1 = minimal; 2 = mild; 3 = moderate; and 4 = intense irritation. This scoring system correlates to human irritation potential as follows: scores of 0 to 8 are acceptable; scores of 9 to 10 indicate borderline irritation potential; and scores of 11 and above are indicative of significant irritation potential.

Statistical Evaluation of Data

Numerical differences in the proportion of pregnant rabbits and implanted embryos between control and Conceival-treated groups were analyzed by Fisher's exact probability test. The statistical significance of the treated group mean with that of control group was analyzed using the 1-way

Table 2. Components of Conceival*

Ingredients	Conceival (% wt/wt)
Sorbitol	30–40
PEG-400	20–23
Polysorbate 80	2–4
Microcrystalline cellulose	0.35–0.45
Xanthan gum	0.095–0.105
Water	32–48

*PEG indicates polyethylene glycol.

analysis of variance, followed by Dunnett's multiple comparison test using GraphPad Prism (Version 4.0a) software (San Diego, CA). Differences were considered statistically significant if P was less than .05.

RESULTS AND DISCUSSION

Solubility and Formulation Studies

PHI-346 and PHI-443 were practically insoluble in water (< 0.001 mg/mL), whereas the solubility of PHI-113 was 3 mg/mL (Table 1). The octanol-water partition coefficient for PHI-113, PHI-346, and PHI-443 were 1.21, 4.01, and 4.39, respectively. These high octanol-water partition coefficient (log P) values suggested good solubility in lipophilic solvents.

Solubility of PHI-113, PHI-346, and PHI-443 was evaluated in 11 different solvent systems. In 5 (ie, Cremophor EL, ethanol, PEG 300, polysorbate 80, and propylene glycol) of the 10% cosolvent systems tested, solubility of PHI-113 ranged from 4.6 to 12 mg/mL. In nonaqueous water miscible excipients (PEG and propylene glycol), the solubility was greater than 20 mg/mL (Table 1). Solubility of PHI-346 in 6 (ie, Cremophor EL, Labrasol, propylene glycol, polysorbate 80, Solutol HS, and vitamin E TPGS) of the 10% cosolvent systems tested ranged from 0.4 to 1.2 mg/mL. The solubility of PHI-443 in 5 (ie, Cremophor EL, Gelucire, Labrasol, polysorbate 80, and vitamin E TPGS) of the 10% cosolvents tested ranged from 0.14 to 0.27 mg/mL. The solubility of PHI-443 in ethanol and oleic acid was 1.65 and 4.5 mg/mL, respectively. In nonaqueous water miscible excipients, the solubility was 28 mg/mL in PEG and 18.2 mg/mL in propylene glycol (Table 1). In accordance with these solubility profiles, several formulations were prepared and screened.

The solubility values for these anti-HIV drugs with admixtures of varying amounts of water in hydroxylic excipients showed an exponential rather than a linear relationship with increasing amounts of excipients. These solubility studies indicated that formulations containing 1% to 2% active pharmaceutical ingredients are feasible by adjusting the ratios of the excipients for preclinical and clinical studies.

Various surfactants, water-soluble polymers, and excipients were screened for drug excipient compatibility, gel stability over temperature ranges in the formulation, and the potential for drug precipitation as well as the ability of the formulation to disperse in aqueous environment. Based on these preformulation observations, a lipophilic nonspermicidal gel formulation (viz Conceival) composed of microcrystalline cellulose (Avicel 591), xanthan gum (Xantural 75), and sorbitol as suspending agents with PEG 400 and water as a carrier and polysorbate-80 as the surfactant was optimized (Table 2). The formulation was a solid dispersion of the active ingredient in the formulation matrix with a viscosity at 25°C of 450 to 500 cps. The pH of the final gel formulation was 6.1 (range 6.5-7.0). Conceival enhanced the solubility of poorly water-soluble (<0.001 mg/mL) anti-HIV drugs, PHI-346 and PHI-443, by at least 270- (0.27 mg/mL) and 150-fold (0.15 mg/mL), respectively. The formulated drugs rapidly disperse into the dissolution medium at 37°C. For PHI-113, more than 90% drug release in 5 minutes was evident in synthetic vaginal fluid under sink conditions.

Conceival was highly stable for at least 6 months at 2°C to 8°C and at ambient temperature for up to 3 months. Under these conditions, less than 5% thermal degradation of the anti-HIV drugs was noted as determined by an analytical HPLC. In the Sander-Cramer assay, progressive human and rabbit sperm motility (30%-40%) was evident even after 15 to 30 minutes of exposure of fresh semen to Conceival. By contrast, a 2% N-9-containing gel (Gynol II) completely immobilized all sperm in human or rabbit semen in less than 20 seconds.

Conceival Does Not Impede Pregnancy

In Experiment 1, ovulating NZW rabbits in subgroups of 18 and 20 with and without intravaginal administration of Conceival were artificially inseminated with fresh semen. Fertility was assessed based on the proportion of does with uterine embryo implants on postinsemination day 8 as well as by the ratio of the number of uterine embryos in both uterine horns per number of corpora lutea in the ovaries. The cumulative pregnancy rates and day-8 embryo implantation data are summarized in Table 3. In vivo exposure of sperm to Conceival at the time of AI had no effect on reproductive indices as assessed by the number of embryos (vehicle control 152/222 vs Conceival-treated 107/180), mean number of embryos/litter, or the percentage conceptus (68.5% vs 59.4%, respectively) based on number of embryos to corpora lutea. The total numbers as well as the mean number of ovarian corpora lutea on postovulation/insemination day 8 were similar in both subgroups. In addition, the preimplantation losses in rabbits inseminated with semen exposed to Conceival at the time of AI were

Table 3. Effect of Intravaginal Application of Conceival at the Time of Artificial Insemination on Reproductive Parameters in NZW Rabbits

Parameters	Untreated Control	Conceival
No. of does inseminated[*†]	20	18
No. of females pregnant (%)	18 (90)	16 (83)
Total number of corpora lutea[‡]	222	180
Mean number of corpora lutea	11 ± 2 (range, 8-15)	10 ± 2 (range, 6-15)
Total number of embryos	152	107
Number of embryos/litter	8 ± 2 (range, 5-12)	7 ± 2 (range, 2-11)
Percentage embryos (%)	68.5	59.4
Preimplantation loss (%)[§]	22.4	27.9

[*]Virgin does were artificially inseminated with 0.5 mL of pooled fresh semen (>15 × 10^7 sperm) 15 minutes following the intravaginal administration of 2 mL of Conceival.
[†]Does were induced to ovulate by an intravenous injection of 100 IU of hCG prior to artificial insemination.
[‡]The number of ovarian corpora lutea and implanted embryos were counted on day 8 of pregnancy.
[§]Preimplantation loss = [(number of corpora lutea - number of embryos)/number of corpora lutea] × 100.

not greater than those of untreated control group (22% vs 28%, respectively). These findings indicated preservation of in vivo sperm function despite exposure to Conceival.

In Experiment 2, ovulated NZW rabbits in 3 subgroups of 20, 22, and 25 were intravaginally administered with 0, 1 mL, and 2 mL Conceival followed by AI and allowed to complete term pregnancies. The litter size, as well as the weight and condition of each offspring at birth, was recorded. The pups were allowed to remain with the dam until day 5. The number of pups per litter and the mean weight of the pups surviving on day 5 were used to measure the perinatal effects of Conceival. The reproductive parameters are summarized in Table 4. Despite the in vivo exposure of semen to Conceival, no statistically significant differences in pregnancy rates were apparent between the control and treatment groups. In the control group, 17 out of 20 (85%) rabbits artificially inseminated became pregnant and delivered a total of 121 newborn rabbits. Similarly, 16 out of 22 (73%) rabbits given 1 mL Conceival prior to AI became pregnant ($P > .05$, Fisher's exact test) with a total of 100 newborn pups. In rabbits given 2 mL of Conceival, 13 out of 25 (52%) became pregnant ($P < .05$, Fisher's exact test) and delivered 96 newborn rabbits. When compared with control, intravaginal administration of Conceival at a dilution of 1:3 or 1:6 resulted in 14% and 39% inhibition of fertility, respectively. This reduction in fertility noted at a 1:6 ratio was a result of in vivo semen dilution. Thus, Conceival is not a conception-inhibiting gel. Rabbits that delivered litters following a single intravaginal application of Conceival prior to AI had healthy offspring with no apparent peri- or postnatal repercussions (Table 4). Control and Conceival-treated females that sired pups had no treatment-related differences in mean litter size, survival rate, or mean pup weight on lactation day 2 and day 5. These findings confirmed that intravaginal exposure of sperm to Conceival had no adverse effects on pregnancy rates, pup survival, or early postnatal development.

Table 4. Effect of Intravaginal Application of Conceival Before Artificial Insemination on Fertility, Fetal Survival, and Pup Development

		Conceival:Semen Ratio	
Reproductive Parameters	**Untreated Control**	**1:3**	**1:6**
No. of does per group*	20	22	25
Pregnancy rate (%)	17/20 (85)	16/22 (72.7)	13/25 (52) [†]
Total number of pups	121	100	96
Litter size	7 ± 3	6 ± 3	7 ± 2
Number of viable litters (%)	17 (100)	16 (100)	13 (100)
Pup survival on day 1 (%)	115/121 (95.0)	92/100 (92.0)	85/96 (88.5)
Pup weight on day 1 (g)	not determined	53.6 ± 11.6	52.6 ± 11.6
Pup survival on day 2 (%)	95/115 (82.6)	83/92 (90.2)	77/85 (90.5)
Pup weight on day 2 (g)	67.0 ± 19.3	53.6 ± 11.6	60.1 ± 12.6
Pup survival on day 5 (%)	83/115 (72.2)	58/92 (63.0)	51/85 (60.0)
Pup weight on day 5 (g)	92.2 ± 22.8	92.4 ± 21.7	82.1 ± 18.3

*Aliquots (0.5 mL) of fresh, pooled semen obtained from fertile bucks were used to artificially inseminate the does 15 minutes following intravaginal application of either 1 mL or 2 mL of Conceival. Does were induced to ovulate by an intravenous injection of 100 IU of hCG prior to artificial insemination and allowed to complete term pregnancy (31 ± 2 days).
[†]P .05 vs untreated control.

Table 5. Mean Vaginal Irritation Scores for New Zealand White Rabbits Given Conceival or N-9 Gel Intravaginally for 14 Consecutive Days

Vaginal Component (Range of Possible Score)*	Conceival (n = 9)			4% N-9 (n = 3)		
	CV	MV	UV	CV	MV	UV
Epithelial ulceration (0-4)	0	0	0	3 ± 1	3 ± 2	4 ± 1
Leukocyte infiltration (0-4)	1 ± 1	1 ± 1	1 ± 1	3 ± 1	3 ± 1	4 ± 1
Edema (0-4)	1 ± 1	1 ± 1	0	2 ± 1	2 ± 1	2 ± 1
Vascular congestion (0-4)	1 ± 0	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1
Total score (0-16)	**3**	**3**	**2**	**9**	**9**	**11**

*Mean ± SD values for 9 (Conceival) or 3 (4% N-9) rabbits representing the cervico-vagina (CV), mid-vagina (MV), and uro-vagina (UV). Semiquantitative scoring criterion adapted from Eckstein et al.[32] Individual score: 0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = intense. Correlation to human irritation potential: total score 8 acceptable, 9 to 10 marginal, and ≥11 unacceptable.

Conceival is Nonirritating to Vaginal Mucosa

Table 5 lists the irritation scores for the cervico-vaginal (CV), mid-vaginal (MV), and uro-vaginal (UV) regions separately to reflect potential changes across the regions from the stratified squamous to columnar epithelium, since these regions present different barriers and tend to vary in their sensitivity to mucosal irritants. Despite the high sensitivity of the rabbit vagina to mucosal irritants, in the 14-day intravaginal exposure to Conceival, no adverse clinical signs of mucosal toxicity were evident. Unlike N-9-treated rabbits, which induced marked epithelial ulceration and leukocyte influx (mean individual scores of 3–4 out of 4; total score 9–11 out of 16), none of the 9 rabbits given daily intravaginal application of Conceival developed epithelial ulceration, leukocyte influx, edema, or vascular congestion characteristic of inflammation (mean individual scores 0–1 out of 4; total score 2–3 out of 16). Figure 1 shows the representative vaginal sections of a

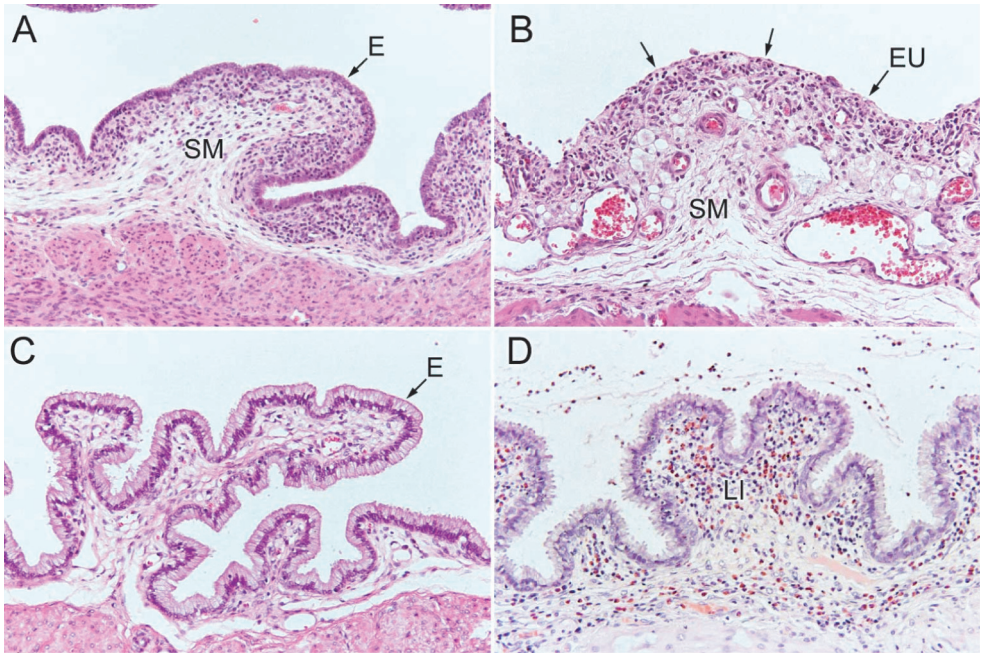


Figure 1. Light microscopy images of Conceival-treated rabbit vaginal sections. Representative hematoxylin (H)- and eosin (E)-stained, paraffin-embedded sections of the mid-vaginal region of a rabbit treated intravaginally with Conceival or N-9 for 14 consecutive days. Left panels: Histology of H- and E-stained upper (A) and lower (C) vaginal sections of a Conceival-treated |rabbit. Right panels: Upper (B) and lower (D) vaginal sections of an N-9-treated rabbit. Note the intactness of vaginal epithelium (E) and lack of leukocyte influx in the submucosa (SM) of Conceival vs N-9-treated rabbit, which shows epithelial ulceration (EU) as well as leukocyte influx (LI) characteristic of inflammation. Original magnification ×200.

rabbit given Conceival or N-9. Light microscopic examination revealed intact vaginal epithelium and lack of leukocyte infiltration in the representative upper and lower regions of vaginal tissues of a rabbit following daily intravaginal administration of Conceival (Figure 1A and C; total score 2). In contrast, vaginal sections of an N-9-treated rabbit (Figure 1B and D) revealed disruption of the epithelial lining and an inflammatory response with influx of leukocytes (total score 10; marginal irritation). Since a correlation exists between rabbits and humans with respect to the irritation potential of vaginal products, these findings indicated that the irritation potential of Conceival is well below the acceptable range (total score = $\leq$8) for clinical trial. Thus, unlike the currently used nonionic or cationic detergent microbicidal spermicides, Conceival is not likely to cause mucosal toxicity following repetitive intravaginal application in humans.

The effectiveness of a vaginal microbicide is dependent on the bioadhesion of the formulation and the bioavailability of the drug. The characteristic feature of Conceival is its unique ability to disperse rapidly on contact with aqueous vaginal environment to bring about self-emulsification or dispersion. The vaginal cavity exhibits an aqueous environment containing secreted glands whose fluids create an acidic pH in the range of 4.0 to 5.5. The formulated anti-HIV drugs in Conceival are rapidly released in aqueous vaginal fluid, thereby facilitating good dispersibility of the drug(s) especially during coitus. Nearly any water-insoluble drug may be formulated in Conceival as to increase its solubility, and hence its bioavailability as a vaginal microbicide without contraceptive activity.

CONCLUSION

Conceival is a novel nontoxic, nonspermicidal, self-emulsifying gel with improved solubility of lipophilic anti-HIV drugs. Conceival has potential to become a clinically useful safe noncontraceptive vaginal vehicle for prophylactic microbicides.

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