Aryl Phosphate Derivatives of Bromo-Methoxy-Azidothymidine Are Dual-Function Spermicides with Potent Anti-Human Immunodeficiency Virus

Osmond J. D’Cruz,1,2,3 T.K. Venkatachalam,2,4,5 Zhaohai Zhu,2,4,5 Mei-Jue Shih,5 and Fatih M. Uckun1,2,5

Drug Discovery Program,2 Departments of Reproductive Biology,1 Chemistry,1 and Virology,5 Wayne Hughes Institute, St. Paul, Minnesota 55113

ABSTRACT

Detergent-based vaginal microbicides, in addition to their high contraceptive failure rates, cause mucosal erosion and local inflammation that might increase the risk of heterosexual human immunodeficiency virus (HIV) transmission. In a systematic effort to identify a microbicide contraceptive potentially capable of preventing the sexual transmission of HIV as well as providing fertility control, a series of novel aryl phosphate derivatives of 5-bromo-6-methoxy-3’-azido-3’-deoxythymidine (AZT; zidovudine) were synthesized and examined for dual anti-HIV and sperm-immobilizing activity (SIA). Whereas AZT displayed potent anti-HIV activity (IC50 = 0.006 μM) but lacked SIA (EC50 > 300 μM), two 5-bromo-6-methoxy-aryl phosphate derivatives of AZT, compounds WHI-05 and WHI-07, exhibited potent anti-HIV activity as well as SIA. The IC50 (HIV) and EC50 (SIA) values for WHI-07 were 439-fold and 13.5-fold lower, respectively, than those for the detergent-based virucidal spermicide, nonoxynol-9 (N-9). Sperm motion kinematics using computer-assisted sperm motion analysis combined with confocal laser scanning microscopy, high-resolution low-voltage scanning, and transmission electron microscopy demonstrated that both WHI-05 and WHI-07 cause a complete and irreversible loss of sperm motility in a concentration- and time-dependent fashion without concomitantly affecting the sperm acrosomal membrane integrity. In experiments designed to assess the fertilizing capacity of treated sperm, preincubation of sperm with either compound resulted in a concentration-dependent loss of the ability to adhere to and penetrate zona-free hamster eggs as well as inhibition of binding to human zona. WHI-07 applied intravaginally prior to artificial insemination of epididymal sperm drastically reduced fertility in hormonally primed CD-1 mice. Unlike the intravaginal application of N-9, repetitive intravaginal application of WHI-07 did not damage the vaginal epithelium or cause local inflammation. Structure-function relationship analyses showed that the addition of bromo-methoxy functional groups to AZT was essential for, and the aryl phosphate derivatization contributory to, the SIA of both compounds. Compounds WHI-05 and WHI-07 may be useful as dual-function vaginal contraceptives for women who are at high risk for acquiring HIV/acquired immunodeficiency virus syndrome by heterosexual vaginal transmission.

INTRODUCTION

Sexually active women represent the fastest growing human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) risk group [1–3]. Heterosexual transmission accounts for > 75% of all HIV infections worldwide and constitutes a growing proportion of new HIV infections in the United States [4, 5]. In the absence of an effective prophylactic anti-HIV vaccine or antiretroviral therapy, female-controlled vaginal microbicides for curbing the mucosal and perinatal HIV transmissions are sought. Nonoxynol-9 (N-9) is the only topical microbicide currently under consideration for protection against sexually transmitted HIV infection in women [6]. This detergent-type virucidal spermicide has been widely used for more than 30 years in over-the-counter gels, foams, creams, and films designed to kill sperm [7, 8]. Although N-9 exerts both spermicidal and antibacterial/antiviral activities against pathogens responsible for sexually transmitted diseases (STD) including HIV in vitro [9–13], recent large scale in vivo clinical trials showed the inefficacy of N-9 to protect from HIV and other STD transmission [14, 15]. In addition, because of its membrane-disruptive properties, this vaginal spermicide has been shown to damage the cervicovaginal epithelium [16, 17], cause an acute tissue inflammatory response [18, 19], and enhance the likelihood of HIV infection by heterosexual transmission [20]. Therefore, new, effective, safe, and female-controlled topical microbicides are urgently needed. In a systematic effort to identify a virucidal spermicide potentially capable of preventing the sexual transmission of HIV as well as providing fertility control, we synthesized a series of novel aryl phosphate derivatives of the anti-HIV agent, 3’-azido-3’-deoxythymidine (AZT; zidovudine) with 5-bromo and 6-methoxy functional groups and examined them for dual anti-HIV and spermicidal activity.

Here, we show that aryl phosphate derivatives of 5-bromo-6-methoxy-AZT are spermicidal and that they maintain potent anti-HIV activities. We have investigated further as to their effects on sperm membrane ultrastructure, interaction of sperm with human and zona-free hamster eggs, local toxicity to the cervicovaginal epithelium, and in vivo contraceptive efficacy of the lead compound, WHI-07, in a mouse model. Our data suggest that AZT derivatives that have 5-bromo-6-methoxy functional groups on the thymine ring may be useful as topical microbicides capable of preventing the sexual transmission of HIV as well as providing fertility control for women who are at high risk for acquiring HIV by heterosexual vaginal transmission.

MATERIALS AND METHODS

Chemical Synthesis of AZT Derivatives

We synthesized three new analogues of AZT (WHI-01, -02, and -03) with 5-bromo and 6-methoxy groups on the thymine ring and azido/amino substitution in the C-3 position of the pentose ring using published procedures [21]. The chemical compositions of these three AZT analogues are shown in Figure 1. Similarly, 9 novel aryl phosphate derivatives of AZT (WHI compounds 04, 05, 06, 07, 08, 09, 10, 11, and 12) were prepared using phosphorochloridate chemistry [22, 23]. In brief, reaction of appropriately substituted phenols with phosphorous oxy chloride in the presence of triethylamine yielded the phosphorochloridate...
derivative. Condensation with AZT in anhydrous tetrahydrofuran containing N-methylimidazole as base yielded the target aryl phosphoramidate derivative of AZT. Further modifications in the structure were achieved by reaction with bromine in anhydrous methanol to obtain the desired target compounds (Fig. 2). For each compound, the purity was > 95%, as determined by the proton (1H) nuclear magnetic resonance spectra (Varian 300 MHz; Varian Associates, Palo Alto, CA) and HPLC. For each compound, the characterization included carbon (13C) NMR, Fourier transform infrared spectra (FT-Nicolet model Protege 460; Nicolet Corporation, Madison, WI), UV-visible spectra (Beckman model DE 7400 spectrophotometer; Beckman Instruments, Fullerton, CA), HPLC (Hewlett Packard model 1100 series; Hewlett-Packard Co., Wilmington, DE), and mass spectra (Hewlett Packard Matrix Assisted Laser Desorption Time-of-Flight spectrometer model G2025A) analysis. Azidothymidine/zidovudine was purchased from Aldrich Chemical Co. (Milwaukee, WI).

HIV-1 Replication Assay

To evaluate anti-HIV-1 activities of AZT, WHI compounds, and N-9, peripheral blood mononuclear cells (PBMC) from HIV-1-negative donors were cultured for 72 h in RPMI 1640 medium (Gibco-BRL, Grand Island, NY) supplemented with 20% (v:v) heat-inactivated fetal calf serum, 5% interleukin-2, 2 mM glutamine, 25 mM Hepes, 2 g/L NaHCO₃, 50 µg/ml gentamicin, and 4 µg/ml phytohemagglutinin prior to exposure to HIV-1 at a multiplicity of infection of 0.1 during a 1-h adsorption period. Subsequently, cells were cultured in 96-well microtiter plates (100 µl/well; 2 × 10⁶/ml, triplicate wells) in the presence and absence of various concentrations (0.001–100 µM) of drugs for 7 days. Aliquots of culture supernatants were removed from the wells on the 7th day after infection for p24 antigen enzyme immunoassay as an indication of viral replication [24, 25]. Percentage inhibition of virus replication was calculated by comparing the p24 antigen values from the test drug-treated infected cells with p24 antigen values from untreated infected cells. The anti-HIV activity of tested compounds was expressed as the IC₅₀ values (the final concentration of the compound in culture medium that decreases the proportion of motile sperm by 50%).

To test the effect of duration of incubation on SIA in the presence of WHI compounds (01, 03, 05, and 07), a motile fraction of sperm (10⁷/ml) was incubated at 37°C in 0.5 ml of BWV-0.3% BSA in the presence of 200 µM WHI-01, or 100 µM each of WHI-03, WHI-05, WHI-07, and N-9 or 1% DMSO alone. At timed intervals, duplicate aliquots (4-µl) were transferred to two 20-µm Microcell (Conception Technologies, San Diego, CA) chambers, and sperm motility was assessed by CASA. For WHI-01, CASA was performed every 20 min for 150 min; for N-9, every 15 min for 120 min; for WHI-05, every 10 min for 70 min; and for WHI-03 and WHI-07, every 5 min for 40 min.

The possible effects of seminal plasma on SIA of WHI compounds were studied either in the presence of 10% cell-free seminal plasma in the assay medium or by direct addition (200 µM) of WHI-03, WHI-05, and WHI-07 to dilutions (1:2–1:6) of liquefied donor semen (n = 3) in PBS and incubation at 37°C. After 3 h of incubation, duplicate aliquots (4-µl) were used for CASA.

To assess the persistence of SIA after removal of WHI compounds, pooled motile sperm (5 × 10⁶) were added to 0.5 ml of assay medium in the presence (100 µM) or absence of WHI-01, WHI-05, and WHI-07 in 1% DMSO. After incubation for 30 min at 37°C, duplicate aliquots were used for sperm motility assessment using CASA. The remaining sperm suspension was washed by the addition of fresh assay medium and centrifugation (500 × g for 5 min). This supernatant was discarded, and the pellet was resuspended in fresh medium (without WHI compounds) to the original volume and reincubated. After 30 min at 37°C, duplicate aliquots were reassessed for sperm motion parameters by CASA. The results were expressed as the mean of two assessments and were compared to the sperm motion parameters of similarly processed sperm suspensions of motile sperm suspended in medium containing DMSO-only controls.

Sperm Kinematic Parameters

For CASA, 4 µl each of sperm suspension was loaded into two 20-µm Microcell chambers placed onto a counting chamber at 37°C. At least 5–8 fields per chamber were scanned for analysis using a Hamilton Thorne Integrated Visual Optical System (IVOS) version 10 instrument (Hamilton Thorne Research Inc., Danvers, MA). Each field was recorded for 30 sec. The Hamilton Thorne computer calibrations were set at 30 frames at a frame rate of 30 images/sec. 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 at 10 µm/sec and...
threshold straightness at 80%; magnification factor, 1.95. The performance of the analyzer was periodically checked using the playback function.

The attributes of sperm kinematic parameters evaluated included numbers of motile (MOT) and progressively (PRG) motile sperm; curvilinear velocity (VCL: a measure of the total distance traveled by a given sperm during the acquisition divided by the time elapsed); average path velocity (VAP; the spatially averaged path that eliminates the wobble of the sperm head), straight-line velocity (VSL; the straight-line distance from beginning to end of track divided by time taken), beat-cross frequency (BCF; frequency of sperm head crossing sperm average path), the amplitude of lateral head displacement (AH; the mean width of sperm head oscillation), and the derivatives, straightness (STR = VSL divided by VAP × 100), linearity (LIN = VSL divided by VCL × 100, departure of sperm track from a straight line). Data from each individual cell track were recorded and analyzed. At least 200 motile sperm were analyzed for each aliquot sampled.

Membrane Integrity Studies

The percentages of sperm with an intact acrosome were evaluated by fluorescence microscopy (Olympus BX-60 model; Olympus Corp., Lake Success, NY) after fixation and ethanol permeabilization of the sperm pellets and subsequent staining with fluorescein (FITC)-conjugated Pisum sativum lectin (Sigma). In positive control sperm, the acrosome reaction was induced by incubating (3 h at 37°C) with 100 µM of calcium ionophore (CaI) A23187 (Sigma). Motile sperm (5 × 10^6/ml) were incubated for 4 h at 37°C in the presence and absence of 100 µM each of WHI-01, WHI-03, WHI-05, WHI-07, or N-9 followed by addition of propidium iodide (PI; 10 µg/ml) and analyzed by flow cytometry. The percentage of sperm positive for PI was determined by flow cytometry using a fluorescence-activated cell sorting Vantage flow cytometer (Becton Dickinson and Co., Mountain View, CA). The sperm in the swim-up fractions were identified by their characteristic forward and 90° angle light-scattering properties [28]. All analyses were done using the 488-nm excitation from a krypton/argon laser with a 635-nm band pass filter for PI emission. The percentage of sperm positive for PI staining was determined using cutoff signals for membrane-intact motile sperm.

Confocal Laser Scanning Microscopy

Ethanol-permeabilized and air-dried sperm smears were stained sequentially with the three fluorescent markers, FITC-Pisum sativum, TOTO-3 iodide, and Nile red (Molecular Probes, Eugene, OR) because their targets are different (acrosome, nucleus, and plasma membrane of permeabilized sperm, respectively). Samples were examined under a Bio-Rad MRC-1024 Laser Scanning Confocal Microscope (Bio-Rad Laboratories, Hercules, CA) equipped with a krypton/argon mixed gas laser (excitation lines 488, 568, and 647 nm) and mounted on a Nikon Eclipse E800 (Garden City, NY) series upright microscope. The fluorescence emissions of fluorescein, TOTO-3 iodide, and Nile red from the acrosomal region, nucleus, and the plasma membrane of sperm after ethanol permeabilization were simultaneously detected using the 598/40 nm, 522 DF32, and 680 DF32 emission/filter, respectively. Confocal images were obtained using a Nikon ×60 (NA 1.35) objective lens and Kalman collection filter. Digitized images were saved on a Jaz disk (Iomega Corporation, Roy, UT) and processed with the Adobe Photoshop software (Adobe Systems, Mountain View, CA). Final images were printed using a Fujix Pictography 3000 (Fuji Photo Film Co., Tokyo, Japan) color printer.

High-Resolution Low-Voltage Scanning Electron Microscopy (HR-LVSEM)

HR-LVSEM was utilized for topographical imaging of different membrane domains over the sperm head as described previously [29, 30]. Aliquots (20 × 10^6) of motile sperm were incubated with DMSO alone (1%), or 100 µM each of WHI-05 or WHI-07 or 10 µM CaI in DMSO for 3 h at 37°C. Washed sperm suspensions were placed on 0.1% poly-L-lysine-coated glass chips and allowed to adhere to the glass over a 60-min incubation period on ice. The supernatants were decanted, and adherent cells were fixed in 1% paraformaldehyde and 1% glutaraldehyde in 0.14 M sodium cacodylate buffer for 3 h. To preserve plasma membrane integrity, sperm were postfixed in 1% osmium tetroxide (OsO4) containing 0.1% ruthenium red in 0.14 M cacodylate buffer for 1 h at 4°C. All samples were dehydrated through an ascending ethanol series, critical point dried, and coated with approximately 2 nm of platinum using ion beam sputtering with argon (4 mA at 10 kV; Ion Tech Ltd., Middlesex, England). All samples were examined in a Hitachi S-900 SEM (Hitachi Instruments, Gaithersburg, MD) at an accelerating voltage of 2 kV. Sperm were observed under low magnification (×2000–5000), and representative sperm were photographed under intermediate magnification (×18 000–25 000). In each specimen evaluated, at least 200 sperm were scanned for the intactness of sperm acrosomal region.

Transmission Electron Microscopy (TEM)

For TEM, treated sperm were fixed in 3% glutaraldehyde in 5% sucrose in 0.2 M cacodylate buffer for 3 h at room temperature. The samples were postfixed in 1% OsO4, 5% sucrose, and 0.1% ruthenium red for 2 h at 4°C. The samples were dehydrated in ascending ethanol series and embedded in Spurr’s epoxy. Thin sections (90 nm) were prepared on a Reichert-Jung (Nossloch, Germany) Ultracut E ultramicrotome (Bretton Dickinson and Co., Mountain View, CA). The sperm in the swim-up fractions were identified by their characteristic forward and 90° angle light-scattering properties [28]. All analyses were done using the 488-nm excitation from a krypton/argon laser with a 635-nm band pass filter for PI emission. The percentage of sperm positive for PI staining was determined using cutoff signals for membrane-intact motile sperm.

Homologous Sperm-Zona Binding Assay

Specifically, human eggs that failed to fertilize in vitro were separately inseminated with a mixture of equal numbers of SYBR 14 (green)- and SYTO 17 (blue)-labeled sperm, the latter pretreated with three concentrations (25, 50, and 100 µM) of the tested compounds. After coinoculation, the number of tightly bound green (SYBR-14) and blue (SYTO-17) sperm were assessed by confocal laser scanning microscopy.

Frozen human eggs (n = 46) were thawed and rinsed three times in BWW-0.3% BSA, and then three times in BWW-3.5% BSA, before addition of sperm suspension. The process of egg recovery, freezing, thawing, and ma-
The fertilizing capacity of human sperm exposed to WHI-05 and WHI-07 was evaluated using the zona-free hamster egg penetration assay [31], with modifications. Motile fractions of sperm (n = 4) obtained by 90–45% Percoll gradient centrifugation and wash were resuspended in BWW-3.5% BSA medium (pH 7.4) and divided into aliquots of 20 × 10^6 sperm/ml and allowed to capacitate for 24 h. Subsequently, sperm were treated at 37°C for 3 h with 25 μM or 100 μM concentrations of WHI-05 and WHI-07 (in 0.5% DMSO) under capacitating conditions. After exposure to drugs, the control and WHI-treated sperm suspensions were washed in BWW-3.5% BSA medium. Sperm motility following capacitation and washing in BWW-3.5% BSA medium was assessed by CASA. Motile sperm labeled with SYBR-14 (green; control) and SYTO-17 blue nuclei were washed thrice in BWW, and one million sperm were added to a dish containing human zona (n = 2) under mineral oil and coincubated for 4 h. After extensive washing of eggs to dislodge any loosely adherent sperm, the eggs were mounted on glass slides and examined under a Bio-Rad MRC-1024 Laser Scanning Confocal Microscope as described above. Using fluorescence imaging, the fluorescence emission of SYBR-14 and SYTO-17 localized on the sperm head was simultaneously recorded using 598/40 nm and 680 DF32 emission/filter, respectively. Confocal images of Z-sections were obtained using a Nikon ×20 (NA 1.4) objective and Kalman collection filter. Digitized images were processed as described above. The number of tightly bound green- and blue-colored sperm nuclei adherent to each human egg was counted from the Z-sections of fluorescence images of each egg on all focal planes and was expressed as a binding ratio.

Heterologous Sperm-Egg Binding and Penetration Assay

Sexually mature adult female CD-1 mice were superovulated by a i.p. injection of 5 IU of eCG (Gestyl; Diosynth B.V., Oss, Holland) followed by an i.p. injection of 5 IU of hCG (Steris Laboratories, Phoenix, AZ) 46–48 h later. Mice were randomly assigned to one of the two treatment groups (27 or 24 per group): 1) vehicle controls received cream base (Taro Pharmaceuticals, Hawthorne, NJ) with 1% DMSO; 2) the test group received cream base with 1% WHI-07 in 1% DMSO. These treatments were given intravaginally (50 μl) prior to artificial insemination. Caudae epididymal sperm were obtained from proven breeder adult CD-1 males [33] and suspended in a modified Krebs-Ringer-bicarbonate medium (M2; Gibco-BRL) supplemented with pyruvate, lactate, and glucose. Sperm were suspended in M2 medium-5% BSA for 1 h prior to use. For each experiment, cauda epididymal sperm pooled from 25 male mice and adjusted to 1–2 × 10^6 motile sperm/50 μl were used for insemination. A 50-μl volume per mouse was ejected through a 1-ml syringe with a blunted 18-gauge needle. Preparations were analyzed for sperm concentration, motility, and sperm motion parameters by CASA using software designed for oval sperm head morphology (Hamilton Thorne). On Day 8, individual females representing the control and test group were killed, and their uteri were examined for the presence or absence of embryos. A total of three independent fertility experiments were performed.

Indirect Immunofluorescence Assay (IFA)

Thirty adult female CD-1 mice were randomly divided into two groups (A and B). Each group was further divided into three subgroups of 5 mice. Mice from group A were treated for 5 days and mice from group B for 20 days via daily intravaginal application of a cream base (Taro Pharmaceuticals) supplemented with 1% DMSO alone (control), 5% WHI-07 in 1% DMSO, or 5% N-9. After 5 and 20 days, respectively, mice were killed by cervical dislocation, and tissues from the genitourinary tract were fixed in 10% buffered formalin. To determine the degree of inflammation and membrane integrity of squamous epithelia, conventional paraffin-embedded sections (6-μm) were prepared and stained with hematoxylin and eosin and observed under ×300 magnification with a Leica (Milton Keynes, Buckinghamshire, UK) microscope interfaced with an image analysis system. The images were captured using the ImagePro Plus program (Media Cybernetics, Silver Spring, MD) in conjunction with a 3CCD camera (DAGE-MTI Inc., Michigan City, KS), and images were transferred to Adobe Photoshop software for printout. The four stages of the estrous cycle were determined histologically.

Indirect Immunofluorescence Assay (IFA)

IFA and confocal microscopy were used to identify the neutrophil infiltrates in the squamous epithelia of cervicovaginal sections of control, WHI-07-treated, and N-9-treated mice. Cervicovaginal tissue sections were deparaffinized, hydrated through graded ethanol, and immunostained with a rat monoclonal antibody (mAb; Cederlane Laboratories Ltd., Westbury, NY) directed against mouse neutrophils (clone 5120-26.1-110). After antigen retrieval by heat-
SPERMICIDAL AZIDOTHYMIDINE DERIVATIVES

RESULTS

5-Bromo 6-Methoxy-Substituted Analogues of AZT Had Spermicidal Activity

The effects of several AZT analogues and derivatives on human sperm function and HIV replication in HIV-infected human PBMC were examined. Exposure of the highly mobile fraction of human sperm to AZT, which inhibited HIV-1 replication in human PBMC in vitro with an IC₅₀ value of 0.006 μM, did not affect sperm motility even at concentrations as high as 300 μM (Fig. 1). Further, sperm motion kinematics using CASA (Hamilton Thorne version 10) confirmed that AZT treatment did not alter the sperm motion parameters, such as the progressive motility, track speed, path velocity, straight-line velocity, straightness of the swimming pattern, linearity of the sperm tracks, beat-cross frequency, and the amplitude of lateral sperm head displacement. Introduction of a bromo at the 5-position (R₂) and methoxy at the 6-position (R₃) on the thymine base ring of AZT to yield 5-bromo-6-methoxy-5,6-dihydro-AZT (compound WHI-01) resulted in gain of significant spermicidal (as determined by sperm motility assay) function (EC₅₀ = 104 μM) without decreasing the anti-HIV activity (Fig. 1). Replacement of the azide group (R₁) in the pentose ring with an NH₂ group (compound WHI-03) substantially enhanced the spermicidal activity (EC₅₀ = 10 μM) but was not spermicidal (EC₅₀ > 300 μM) (Fig. 1).

FIG. 1. Anti-HIV and spermicidal activity profiles of bromo-methoxy-AZT analogues. The inhibitory effects of the listed compounds on the replication of HIV-1 in human PBMC in vitro were evaluated as described previously [24]. The results are expressed as the IC₅₀ (HIV), i.e., the concentration required to inhibit by 50% the activity of HIV-1 replication as measured by assays of p24 antigen production. SIA was determined by CASA as described in Materials and Methods. EC₅₀ (SIA) values represent the concentration required to decrease sperm motility by 50% as measured from the concentration-response curves using CASA. EC₅₀ values were calculated from three separate experiments.

FIG. 2. Structure-activity relationships affecting the anti-HIV and spermicidal activity of aryl phosphate derivatives of 5-bromo-6-methoxy-AZT. Nine new derivatives of compound WHI-01 (WHI-compounds 4–12) were synthesized by phosphorochloridate chemistry. IC₅₀ values were calculated from the concentration-response curves of decreases in HIV-1 replication in p24 antigen production assays. EC₅₀ values calculated from the concentration-response curves for group B compounds are shown. EC₅₀ values are mean of three independent experiments. NA, not applicable.
Motile sperm were incubated at 37°C in the presence of 200 μM of the assay medium, and the percentage of motile sperm was evaluated by CASA. Each data point represents the mean from three independent experiments. 

There was a linear relationship between incubation time and progressive sperm motility by CASA. Regression analysis of progressive motility against duration of incubation yielded identical coefficients of 0.88 (95% confidence interval: 12–26 min) and 6 min (95% confidence interval: 4–11 min), respectively. Complete sperm immobilization was achieved after 120 min of exposure to N-9, 60 min exposure to WHI-05, and 30 min of exposure to WHI-07. By comparison, the sperm motility in control samples remained unchanged for 3 h.

### Kinetics of Sperm Immobilization

The comparative concentration-dependent effects of AZT, three analogues of AZT, and four aryl phosphate derivatives of AZT on sperm motility were analyzed by CASA (Fig. 3A). At a concentration > 25 μM, WHI-07 abrogated the motility of > 95% of the treated sperm, whereas AZT and its derivatives, WHI-02, WHI-08, and WHI-09 without the 5-bromo-6-methoxy functional groups, were inactive even at 300 μM. Also, the kinetics of sperm immobilization was fast with spermicidal AZT derivatives when compared to that with N-9 at 100 μM (Fig. 3B). There was a linear relationship between incubation time and progressive sperm motility by CASA. Regression analysis of progressive motility against duration of incubation time for WHI-05 and WHI-07 showed positive correlation coefficients of 0.88 (p < 0.005) and 0.84 (p < 0.003). The corresponding times required for 50% motility loss of progressively motile sperm exposed to WHI-05 and WHI-07 (at 100 μM concentration) were 17 min (95% confidence interval: 12–26 min) and 6 min (95% confidence interval: 4–11 min), respectively. Complete sperm immobilization was achieved after 120 min of exposure to N-9, 60 min exposure to WHI-05, and 30 min of exposure to WHI-07. By comparison, the sperm motility in control samples remained unchanged for 3 h.

### Table 1. Effect of brief exposure and sperm washing on spermicidal activity of aryl phosphate derivatives of bromo-methoxy-AZT.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Motility&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
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<td>Prewash</td>
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<td>DMSO (0.1%)</td>
<td>86 ± 3</td>
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<tr>
<td>WHI-05 (100 μM)</td>
<td>17 ± 4</td>
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<tr>
<td>WHI-07 (100 μM)</td>
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<sup>a</sup> Sperm motility was assessed by CASA.

<sup>b</sup> CASA was performed after 30 min of incubation in BWW-0.3% BSA medium without DMSO or drugs.

### FIG. 3. A) Concentration-dependent inhibition of sperm motility by 5-bromo-6-methoxy-AZT derivatives. The highly motile fraction of sperm was incubated for 3 h with increasing concentrations (4.6–300 μM) of AZT, WHI compounds 01, 02, 03, 05, 07, 08, and 09, or 1% DMSO in the assay medium, and the percentage of motile sperm was evaluated by CASA. Each data point represents the mean from three independent experiments. B) Time-dependent sperm immobilization by bromo-methoxy-AZT derivatives. Motile sperm were incubated at 37°C in 0.5 ml of assay medium in the presence of 200 μM of WHI-01 or 100 μM each of WHI-03, WHI-05, WHI-07, and N-9 or 1% DMSO alone. At timed intervals, sperm motility was assessed by CASA. For WHI-01, CASA was performed every 20 min for 150 min; for N-9, every 15 min for 120 min; for WHI-05, every 10 min for 70 min; and for WHI-03 and WHI-07, every 5 min for 40 min. Each data point represents the mean from three independent experiments. In A and B, the SD values were less than 10% of the mean values.
Spermicidal AZT Derivatives Affected Sperm Kinematics

The time-dependent sperm motility loss induced by WHI-01, WHI-05, and WHI-07 was associated with significant changes in the movement characteristics of the surviving sperm, particularly with respect to the track speed (VCL), path velocity (VAP), and straight-line velocity (VSL). The representative sperm kinematic parameters observed for WHI-07-treated sperm versus time are shown in Figure 4. The decreases in VSL and VCL were similar in magnitude. Therefore, linearity (LIN) of the sperm tracks were not altered from 5 to 20 min in the presence of WHI-07, from 10 to 60 min in the presence of WHI-05, and from 20 to 150 min in the presence of WHI-01. Similarly, the decreases in VSL and VAP were similar in magnitude. Therefore, straightness (STR) of the swimming pattern was not altered either. Also, the beat-cross frequency (BCF) and the amplitude of lateral sperm head displacement (ALH) were relatively uniform as the proportion of motile sperm declined with exposure time. The sperm motion parameters of control sperm showed insignificant changes during the 150-min exposure.

Spermicidal Activity of AZT Derivatives Was Irreversible

In order to determine whether the SIA of WHI-05 or WHI-07 was reversible, sperm exposed to 100 μM of either compound for 30 min were washed and resuspended in fresh sperm motility assay medium, and sperm motility was reassessed by CASA (Table 1). No recovery in sperm motility was observed, indicating that the drug-induced sperm immobilization was irreversible. Direct addition of compounds WHI-05 and WHI-07 to semen diluted in PBS resulted in complete loss of motility as assessed by CASA (data not shown). Thus, the spermicidal activity was not affected by seminal plasma.

Lack of Membrane Disruption by Spermicidal AZT Derivatives

Because the great majority of spermicidal compounds are believed to immobilize sperm as a result of a detergent-type action on the sperm plasma membrane [34–36], we also tested the effects of WHI-05 and WHI-07 on sperm plasma membrane permeability by flow cytometric analysis of PI-stained sperm and acrosome integrity by examination of FITC-lectin-, TOTO-3 iodide-, and Nile red-stained sperm using confocal laser scanning microscopy. Despite complete immobilization of sperm in the presence of WHI-05 and WHI-07, <3% of sperm were permeable to PI after 4 h of incubation with these compounds. In acrosome integrity studies, WHI-05- and WHI-07-treated sperm (similar to sham-treated sperm) remained acrosome intact (97 ± 2% and 93 ± 5.6%, respectively) after 3 h of incubation at a concentration of 100 μM despite a complete loss of motility.

Examination of FITC-lectin-, TOTO-3-, and Nile red-stained sperm by confocal microscopy revealed an intense acrosomal staining with FITC-lectin (green), nuclear staining with TOTO-3 (blue), and membrane staining (red) with Nile red, respectively (Fig. 5, upper panels). In acrosome-intact sperm, more than half of the sperm head (the acrosomal region) exhibited a uniform, bright green fluorescence in sperm exposed to vehicle (i.e., 1% DMSO) alone (Fig. 5A), 100 μM WHI-05 (Fig. 5B), and 100 μM WHI-07 (Fig. 5C) for 3 h. By comparison, in sperm exposed to 100 μM of N-9 for 3 h under identical conditions, green fluorescence was absent due to disruption of membrane integrity and acrosomal loss (Fig. 5D), consistent with previous SEM observations [34]. Thus, the spermicidal activity of the dual-function AZT derivatives was not accompanied by a loss of membrane integrity.

Topographical imaging of drug-treated sperm heads by HR-LVSEM revealed intact acrosomes (Fig. 5, middle panels) with smooth contiguous surfaces in sperm exposed to vehicle (Fig. 5A’ or 100 μM WHI-05 (Fig. 5B’), whereas sperm treated with WHI-07 (Fig. 5C’) revealed signs of a
mild acrosomal membrane ruffling. By comparison, sperm exposed to 10 μM Cal A23187, as a positive control, revealed characteristic blebbing or vesiculation, fenestration, and loss of plasma and acrosomal membranes (Fig. 5D’). TEM of the tangential sections of sperm head confirmed the intactness of the plasma membrane and outer and inner acrosomal membranes of sperm exposed to vehicle (Fig. 5A’’), WHI-05 (Fig. 5B’’), and WHI-07 (Fig. 5C’’) but not to the Cal A23187 (Fig. 5D’’) for 3 h (Fig. 5, bottom panels). Taken together, these results demonstrate that the spermicidal effects of WHI-05 or WHI-07 were not caused by a detergent-type action resulting in disruption of the sperm plasma membrane within the acrosomal region of the sperm head. These features of aryl phosphate derivatives of bromo-methoxy-AZT differ from those of N-9 [34], the most widely used prototype detergent spermicide.

Inhibitory Effects of WHI-05 and WHI-07 on Homologous Sperm-Zona Binding Assay

The human sperm-zona recognition and binding assay appears to predict the in vitro and in vivo fertilization outcome [37, 38]. Therefore, the inhibitory effects of WHI-05 and WHI-07 on sperm-egg interaction were next assessed by laser scanning confocal microscopy using intact human zona and two-colored sperm separately labeled with cell-permeant DNA-specific dyes, SYBR 14 (green) and SYTO 17 (blue), in a homologous sperm-zona binding assay. Due to the variable efficiency of sperm binding to frozen human zona, the sperm-zona binding ratio was used to assess sperm-zona binding capacity. The ratios of the number of green and blue sperm that were bound to control and test human zona showed marked differences. The mean sperm-zona binding ratio for control eggs coincubated with SYBR 14- and SYTO 17-labeled control sperm was 1.07. In contrast, the mean sperm-zona binding ratios for eggs coincubated with WHI-05-treated sperm (SYBR 17) were 0.988, 0.039, and 0.009, respectively. The corresponding mean ratios for sperm treated with 25, 50, and 100 μM WHI-07-treated sperm (SYTO 17) were 0.18, 0.07, and 0.10, respectively. Figure 6 shows the representative sperm-zona binding patterns of green and blue sperm to a control egg (Fig. 6A) and the test eggs in which the blue sperm were pretreated with either 100 μM of WHI-05 (Fig. 6B) or 25 μM of WHI-07 (Fig. 6C) prior to coincubation with green-labeled sperm. Despite the variable number of sperm binding to individual zona, a clear reduction in the number of blue-colored sperm bound to test eggs is apparent in comparison to control eggs.

FIG. 5. Upper panels Laser scanning confocal fluorescence images of sperm. Triple labeling of sperm with FITC-Pisum sativum lectin for acrosome (green), TOTO-3 iodide for DNA (blue), and Nile red for membrane lipid (red) incubated in the absence (A) and presence of WHI-05 (B), WHI-07 (C), and N-9 (D) for 3 h. An intense acrosomal staining with FITC-lectin (green), nuclear staining with TOTO-3 (blue), and plasma membrane staining (red) of the sperm tail region with Nile red are apparent. In acrosome-intact sperm, the acrosomal region of the sperm heads exhibited a uniform, bright green fluorescence. In acrosome-reacted sperm, green fluorescence was either absent or restricted to the equatorial segment of the sperm heads. Sperm exposed to 1% DMSO alone (A), WHI-05 (B), and WHI-07 (C) did not reveal increased acrosome reaction at 3 h of incubation. Sperm exposed to 100 μM of N-9 under identical conditions revealed only acrosome-reacted sperm (D) (original magnification ×1000). Middle panels High-resolution low-voltage scanning electron micrographs of sperm incubated in the absence (A’) and presence of 100 μM each of WHI-05 (B’), WHI-07 (C’), and 10 μM Cal (D’) for 3 h (×18 000 magnification). Postfixation with Oso4, and ruthenium red yielded very smooth plasma membrane over the acrosome-intact sperm head. The smooth acrosomal surface is delimitated from the postacrosomal region by an equatorial band (A’). Sperm exposed for 3 h to WHI-05 and WHI-07, respectively, reveal intact acrosome with various degrees of ruffling of the plasma membrane. Cal-treated sperm reveal vesiculation, blebbing, and loss of the plasma and acrosomal membranes and well-preserved postacrosomal membrane. Bottom panels Transmission electron micrographs of sperm incubated in the absence (A’’) and presence of 100 μM each of WHI-05 (B’’), WHI-07 (C’’), or 10 μM Cal (D’’) for 3 h (×18 000 magnification). The plasma membrane is present over the sperm head. Both acrosomal and postacrosomal membranes are clearly visible after 3 h of incubation with WHI-05 and WHI-07. Note the complete loss of acrosome in Cal-treated sperm (D’’). Figure reproduced at 84% of original.

FIG. 6. Laser scanning confocal fluorescence images of two-color human sperm-zona interaction. Motile sperm were labeled separately with cell-permeant DNA-specific dyes, SYBR 14 (green) or SYTO 17 (blue), combined in equal proportion and coincubated with zona-intact human eggs with or without prior treatment of SYTO 17-labeled sperm with 25, 50, and 100 μM of WHI-05 or WHI-07. A) Binding of two-colored untreated control sperm (SYBR 14 + SYTO 17) to human zona of a control egg. The zona pellucida is not visible with the fluorescent optics. B) Binding of SYBR 14-labeled untreated and SYTO 17-labeled WHI-05-treated (100 μM) sperm to human zona of a test egg. C) Binding of SYBR 14-labeled untreated and SYTO 17-labeled WHI-07-treated (25 μM) sperm to human zona of a test egg. Note the binding of both green and blue sperm to control egg and the predominance of green sperm binding to test eggs. The number of sperm bound to individual zona is variable (original magnification ×200).
Inhibitory Effects of WHI-05 and WHI-07 on Heterologous Sperm-Zona-Free Hamster Egg Binding and Penetration Assay

The heterologous zona-free hamster egg penetration assay appears to correlate with the in vivo fertilizing capacity of human sperm as well as the ability of human sperm to fertilize intact human eggs in vitro [39, 40]. Therefore, the inhibitory effects of WHI-05 and WHI-07 on sperm-egg interaction were further confirmed using the zona-free hamster egg penetration assay. As expected, preincubation of capacitated sperm with either compound resulted in significant inhibition (p < 0.001) of sperm binding to zona-free eggs (Table 2). Similarly, the penetration rate of human sperm to zona-free hamster eggs following exposure to increasing concentrations of WHI-05 or WHI-07 showed marked inhibition (41–100% inhibition when compared with control). Thus, pretreatment of human sperm with spermicidal aryl phosphate derivatives of bromo-methoxy-AZT resulted in a concentration-dependent loss of the ability to bind and penetrate zona-free hamster eggs as well as inhibition of binding to human eggs.

In Vivo Contraceptive Efficacy of WHI-07

Because fertilization in vivo is dependent on successful sperm transport through the female genital tract [41], we next determined whether exposure of sperm to the lead compound, WHI-07, in vivo affected the subsequent fertilization outcome. Hormonally primed adult female Swiss (CD-1) mice were artificially inseminated with motile epididymal sperm via the cervix with and without prior intravaginal application of 1% WHI-07 in a cream base. Females were examined 8 days later for the presence or absence of embryos in uteri. The results of three fertility trials are summarized in Table 3. In mice receiving WHI-07 intravaginally prior to artificial insemination, the percentage of fertility rate was drastically reduced in comparison to that in controls (7.6% vs. 38.4%; p < 0.001), which demonstrates a substantially impaired ability of WHI-07-exposed sperm to reach the site of fertilization.

Lack of Local Toxicity of WHI-07

Next, the local tissue alterations and inflammatory response to repetitive intravaginal applications of N-9 versus WHI-07 in mice were compared. Two groups of 15 adult female Swiss (CD-1) mice were treated for 5 (group A) or 20 (group B) consecutive days with either 5% N-9, 5% WHI-07, or control vehicle in a cream base, and the cervicovaginal tissue sections were examined for histopathological changes and influx of inflammatory cells (Fig. 7). The cervicovaginal region consisted of a stratified squamous epithelium overlying a vascular submucosa. The thickness of the epithelium varied depending on the stage of the estrous cycle. Similar to what was observed in the 10 vehicle alone-treated control mice, none of the 10 mice treated with WHI-07 for 5 days or 20 days revealed any inflammatory response or membrane disruption of the squamous epithelia (Fig. 7A and Table 4). By contrast, disruption of the epithelial lining, and an inflammatory response with influx of neutrophils in the squamous epithelia of cervicovaginal crypts, were evident in 9 of 10 mice given N-9 intravaginally (Fig. 7C and Table 4), consistent with previously published observations in rats [19].

Two-color laser scanning confocal fluorescence images of the cervicovaginal epithelia of WHI-07- and N-9-treated sections with a monoclonal antibody specific to mouse neutrophils revealed absence of neutrophils in the stratified squamous epithelial crypts of WHI-07-treated specimens (Fig. 7B) and intense positive staining (green color) in the squamous epithelia of N-9-treated tissue section (Fig. 7D).

Thus, evidence obtained by histopathologic and immunofluorescence studies indicates that unlike N-9 treatment, intravaginal application of WHI-07 in a cream base does not cause any membrane disruption or an acute inflammatory response in the cervicovaginal epithelial crypts.

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TABLE 2. Inhibitory effects of WHI-05 and WHI-07 on human sperm binding and penetration of zona-free hamster eggs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Binding</th>
<th>Penetration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>15.0 ± 14.8</td>
<td>68/121 (56)</td>
</tr>
<tr>
<td>WHI-05 25 μM</td>
<td>5.6 ± 7.0d</td>
<td>7/28 (25)</td>
</tr>
<tr>
<td>WHI-05 100 μM</td>
<td>0.1 ± 0.4d</td>
<td>0/15 (0)</td>
</tr>
<tr>
<td>WHI-07 25 μM</td>
<td>4.1 ± 7.3d</td>
<td>11/88 (12)</td>
</tr>
<tr>
<td>WHI-07 100 μM</td>
<td>5.8 ± 7.0d</td>
<td>10/59 (16)</td>
</tr>
</tbody>
</table>

TABLE 3. Fertility of female mice after artificial insemination of sperm with cervical with and without intravaginal application of WHI-07.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of mice inseminated/group</th>
<th>No. of control mice fertile (%)</th>
<th>No. of WHI-07-treated mice fertile (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>10 (37)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>11 (40)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>9 (37)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>30 (38)</td>
<td>6 (7)d</td>
</tr>
</tbody>
</table>

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TABLE 4. Comparison of local toxicity to cervicovaginal epithelia of mice after repetitive intravaginal application with N-9 or WHI-07 as determined by histopathology.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PMN influx</th>
<th>Disruption of epithelia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 5 days</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>WHI-07 5 days</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>N-9 5 days</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>WHI-07 20 days</td>
<td>4/5</td>
<td>4/5</td>
</tr>
</tbody>
</table>

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*p < 0.001.
FIG. 7.  A–D) Histological and confocal fluorescence images of WHI-07- and N-9-treated mouse cervicovaginal sections. Left) Representative hematoxylin- and eosin-stained, paraffin-embedded sections of the cervicovaginal region of mouse treated intravaginally with 5% WHI-07 (A) or 5% N-9 (B) in a cream base for 20 and 5 consecutive days, respectively. The mucosa consisted of stratified squamous epithelium (SE) over a vascular submucosa. Note the lack of inflammatory infiltrate of neutrophils and the intactness of squamous epithelia in WHI-07-treated sections in contrast to N-9-treated sections (arrow); original magnification: ×200–300. Right) Two-color laser scanning confocal fluorescence images of the cervicovaginal region of mice treated intravaginally with 5% WHI-07 (C) or 5% N-9 (D) for 20 and 5 consecutive days, respectively. Double labeling of the cervicovaginal epithelia with a rat monoclonal antibody to neutrophils and FITC-conjugated anti-rat IgG as the secondary antibody and counterstaining of cell nuclei with PI (red), showed lack of neutrophils (green fluorescence) in WHI-07-treated tissue section, while N-9-treated cervicovaginal epithelia shows clusters (arrow) of brightly green fluorescent neutrophils. L, Lumen; SE, squamous epithelium; original magnification: ×300–400.

DISCUSSION

The development of a new class of aryl phosphate derivatives of bromo-methoxy-AZT as dual-function spermicides with potent anti-HIV activity was reported. CASA based on centroid-derived movement characteristics revealed that the observed spermicidal activity of these novel anti-HIV drugs and prodrugs of AZT was proportional to their effects on the track speed, path velocity, and straight-line velocity of sperm. The concentrations of the lead compound, WHI-07, required for 50% inhibition of HIV replication were 439-fold lower than that for the currently used detergent-based virucidal spermicide, N-9. The spermicidal activity of WHI-07 was 13.5-fold more potent than that of N-9 and was not mediated by membrane disruption. With the most potent AZT derivatives described herein, sperm motility loss was complete within 30 min. Results of our structure-activity relationship studies clearly establish that addition of the bromo-methoxy functional groups was essential for, and the aryl phosphate derivatization contributory to, the spermicidal activity of two classes of AZT derivatives. The SIA of AZT derivatives was irreversible. The potent spermicidal and anti-HIV activity of bromo-methoxy-substituted aryl phosphate derivatives of bromo-methoxy-AZT may provide the basis for the development of new and effective vaginal microbicides.

Physiological fertilization depends on the ability of the ejaculated sperm to swim, bind the zona pellucida, and penetrate the egg, all of which abilities are primarily dependent on sperm motility [41, 42]. The mechanisms by which spermicidal AZT derivatives affect sperm motility are not fully understood; however, their spermicidal activity differed from that of detergent-type spermicides. The fact that spermicidal AZT derivatives were not permeable to PI, as well as the intactness of the plasma membrane and acrosomal membranes observed after several hours of exposure to these drugs, suggested that these spermicidal AZT derivatives immobilize sperm without membrane disruption as confirmed at the ultrastructural level using HR-LVSEM, which reveals the true surface of a cell in its exposed state [29, 30]. These properties of AZT derivatives differ from those of currently used spermicides, N-9 and gramicidin, which exert their effects via a detergent-like ability to damage the sperm membrane, perturb its conformation, and destroy its semipermeable nature, thereby impairing the sperm motility.
motility and egg-fertilizing functions [34–36]. Because of their nonspecific membrane-disruptive properties, such vaginal spermicides have been shown to damage the cervicovaginal epithelium [17–19], as well, which may lead to a lower degree of protection from STD [20]. A vaginal contraceptive that does not elicit any of the nonspecific membrane toxicity characteristic of detergent-type contraceptives would be desirable.

The human sperm-zona recognition and binding assays and the zona-free hamster egg binding and penetration assays are useful in predicting the in vivo fertilizing capacity of human sperm [37–40]. We investigated the effects of dual-function AZT derivatives, WHI-05 and WHI-07, on the membrane fusogenic potential of sperm in the hamster egg penetration assay. Both these AZT derivatives that affected sperm motility also inhibited binding and fertilization of zona-free hamster eggs in a concentration-dependent manner. Furthermore, using a novel two-colored DNA-stained sperm-zona binding competition assay and confocal microscopy, we also demonstrated the concentration-dependent capacity of WHI-05 and WHI-07 treatment to inhibit binding of sperm to human zona as compared with that of control sperm using the same human zona. The use of cell-permeant DNA specific fluorescent dyes SYBR 14 and SYTO 17 to bind zona enhanced the specificity of the human sperm-zona binding test in comparison to conventional two-colored sperm-zona binding assays that employ nonspecific fluorescein- and rhodamine-labeled protein tags to detect fluorescent sperm binding to zona [43, 44].

Fertilization in vivo depends upon successful sperm transport through the female genital tract. Results of our in vivo contraceptive efficacy studies in the mouse model demonstrated that prior exposure of artificially inseminated epididymal sperm to WHI-07 via cervix significantly affected sperm fertilizing ability in vivo in hormonally primed female mice, indicative of the inability of WHI-07-exposed sperm to reach the site of fertilization. The results of this pilot study indicate that WHI-07 would be an attractive candidate for further contraceptive efficacy trials in larger animal models.

Experiments designed to test the comparative local toxicity of N-9 versus WHI-07 clearly demonstrated that, unlike what occurs with the intravaginally applied N-9, repetitive intravaginal application of WHI-07 did not induce an inflammatory response by detergent-type disruption. Evidence obtained by histopathologic and immunofluorescence studies suggests that intravaginal application of N-9 caused marked underlying acute inflammatory response in the cervicovaginal epithelial crypts. The infiltrating leukocytes were identified to be neutrophils. These histopathologic features of N-9 observed in this study are similar to those observed in the rat model [19]. That repetitive intravaginal infusion of WHI-07 does not cause a detergent-type membrane injury or an acute inflammatory response in the cervicovaginal epithelium, as demonstrated by our studies, may prove clinically advantageous. Future preclinical studies will examine intravaginally applied WHI-07 for potential systemic effects, especially those on female genital tract and reproductive function as well as early embryonic development and fetal morphogenesis.

The development of novel aryl phosphate derivatives of bromo-methoxy-AZT with potent spermicidal and anti-HIV activities represents a significant step forward in the development of a new virucidal spermicide for curbing heterosexual HIV transmission. The promising results reported herein warrant the further development of these dual-function AZT derivatives as a new class of vaginal contraceptives capable of preventing the sexual transmission of HIV while providing fertility control for women who are at high risk for acquiring HIV by heterosexual transmission. These dual-function AZT derivatives may also have utility in curbing domestic and wildlife animal retroviral transmissions.

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REFERENCES


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