The current pandemic of sexually transmitted human immunodeficiency virus (HIV) infection—the causative agent of acquired immunodeficiency syndrome (AIDS), has created an urgent need for a new type of contraceptive: one that is both a spermicide and a microbicide. Because most women at risk for HIV infection are of reproductive age (15–44 years), effective use of dual-function contraceptives is important to prevent HIV transmission and unintended pregnancies. In the absence of an effective prophylactic anti-HIV therapy or vaccine, new emphasis has been placed on the development of intravaginal microbicidal agents capable of reducing the transmission of HIV. Topical microbicidal spermicides would ideally provide a female-controlled method of self-protection against HIV as well as preventing pregnancy. However, several microbicides that are undergoing preclinical and human clinical trials contain detergent-type ingredients. The detergent-type spermicide, nonoxynol-9, the only recommended microbicide for protection against sexual transmission of HIV has been shown to cause lesions in vaginal and cervical epithelia leaving women more vulnerable to HIV infection. Therefore, a major challenge in microbicide research has been to design mechanism-based microbicides that are highly effective against pregnancy and HIV transmission while lacking detergent-type effects on epithelial cells and normal vaginal flora. We present an overview of current microbicidal research and report on the identification and preclinical development of novel non-detergent spermicidal nucleoside and non-nucleoside inhibitors aimed at decreasing pregnancy and preventing sexual transmission of HIV.

Key words: AIDS/AZT/HIV/spermicidal contraceptives/viricidal microbicides/ZDV

TABLE OF CONTENTS

Introduction 506
Women and HIV/AIDS 507
Disadvantages of current microbicidal spermicides 507
Properties required of topical microbicides 508
Preclinical and clinical development of microbicides 508
Spermicidal nucleoside analogues 509
Spermicidal non-nucleoside inhibitors 512
Conclusions 512
Acknowledgements 513
References 513

Introduction

For women of reproductive age, a variety of contraceptive options are available, including vaginal spermicides, hormonal contraceptives, intrauterine devices, barrier methods, and tubal ligation. Few of these methods, however, provide effective protection against sexually transmitted diseases (STDs). The decline in oral contraceptive and diaphragm use and the increase in reliance on condoms and spermicides from 1988–1995 suggest that concerns about human immunodeficiency virus (HIV) infection—the causative agent of acquired immunodeficiency syndrome (AIDS) and other STDs, are changing patterns of contraceptive usage among sexually active adolescent and adult women worldwide. Due to the increased incidence of genital herpes, hepatitis B, human papilloma virus, and HIV as well as the increased awareness of the general public regarding AIDS and other STDs, the development of microbicidal spermicides particularly with antiviral activity is a high priority in contraception research (Potts, 1994). Topical microbicidal spermicides are now considered the ‘hope de jour’ because they would ideally provide a convenient, readily available method of self-protection against STDs while preventing pregnancy. Therefore, the goal is to develop a wide range of prophylactic contraceptives that will offer optimal strategies both for fertility control and protection from STDs including HIV. A number of vaginal (and rectal) microbicides are currently undergoing preclinical or human clinical trials to assess their acceptability, safety, and efficacy in protecting women from STD/HIV.
infection. However, a major challenge has been to design mechanism-based microbicides that are highly effective against pregnancy and STD/HIV infection while lacking detergent-type effects on epithelial cells and normal vaginal flora (Elias and Heise, 1994). This review will focus on the development of novel prophylactic contraceptives for HIV prevention.

**Women and HIV/AIDS**

Sexually active women represent the fastest growing AIDS risk group. Worldwide, heterosexual transmission accounts for 90% of all HIV infections in women (CDCP Update, 1994; Celum and Watts, 1996). The world regions where women are most heavily affected by HIV are located in sub-Saharan Africa, Latin America and the Caribbean and Asia, particularly Thailand and India (Keenlyside et al., 1993; Quinn, 1996). Currently an estimated 13.8 million women worldwide are infected with HIV, representing 43% of all adult infections.

In the USA, the proportion of women with AIDS has risen from 7% in 1985 to 22% in 1997. Today, an estimated 120,000–160,000 women are infected with HIV; an estimated 1.5/1000 of these women giving birth are infected with HIV. Also, the incidence of AIDS has increased rapidly among younger people. Of women with AIDS, 85% are aged 15–44 years. HIV infection is the third leading cause of death among American women aged 25-44 years and ranks first among African-American and Hispanic women in this age group (CDCP Update 1996; Ward and Duchin, 1997).

Considering that the AIDS epidemic is still in its infancy on a global scale, this evolving demographic situation warrants urgent attention particularly for the adolescent population. Therefore, effective strategies are needed to reduce heterosexual and perinatal HIV transmission. In the absence of an effective prophylactic anti-HIV therapy or vaccine, new emphasis has been placed on the development of intravaginal microbicidal agents capable of reducing the transmission of HIV (Cookson, 1993; Lange et al., 1993). The development of dual-function vaginal microbicide/spermicides would have a tremendous impact worldwide. Prophylactic contraception is fundamentally important in HIV-infected women for prevention of HIV transmission and pregnancy, especially because 80% of women with AIDS are of childbearing age (CDCP Update, 1997).

**Disadvantages of current microbicidal spermicides**

At present, all commercially available spermicidal microbicides have detergent ingredients that disrupt cell membranes. These include the neutral surfactants: isononyl-phenyl-polyoxyethelene (9) ether (nonoxynol-9; N-9); p-menthanyl-phenyl-polyoxyethelene (8,8) ether or menfegol; and isoctyl-phenyl-polyoxyethelene (9) ether (octoxynol-9; O-9). The detergent-type vaginal spermicide, N-9, available without a prescription, is the most commonly used spermicidal contraceptive in the UK and USA (OTC Panel, 1980; Chantler, 1992). Worldwide, the cationic surfactant benzalkonium chloride, the anionic detergent sodium docusate (dioctyl sodium sulphosuccinate) are also used as vaginal spermicides. The potential candidates for topical use currently available, for example, N-9, sodium oxychlorosene, and benzalkonium chloride, have been used as gels, suppositories, ovules, or sponges. N-9 has been in use for >30 years in concentrations of 2–6% in creams and gels, 12% in foams and up to 18% in condom lubricants. However, in several large studies for users of N-9, the average 6 month pregnancy rate is 26%, and the first year pregnancy rates range from 11 to 31%, making N-9 ~75% effective in preventing pregnancy (Trussell and Trost, 1987; Kulig et al., 1989; Raymond and Dominik, 1999). Additionally, N-9 is only ~38 and 25% effective in preventing gonococcal and chlamydial cervical infections respectively (Cook and Rosenberg et al., 1998). Thus, the dual-protection provided by N-9 is nominal. However, because N-9 was shown to have some potential to inactivate HIV in laboratory tests (Hicks et al., 1985; Polsky et al., 1988), it is the only topical microbicide currently being recommended for protection against sexually transmitted HIV infection in women (Wittkowski, 1995; Elias and Heise, 1995; Cook and Rosenberg, 1998).

The spermicidal activities of these surfactants are associated with their structural affinity to the membrane lipids (Schill and Wolf, 1981; Wilborn et al., 1983). Therefore, the major drawback of using N-9 or other surfactants is their detergent-type effect on epithelial cells and normal vaginal flora. Frequent use of N-9 as a vaginal contraceptive has been associated with an increased risk of vaginal or cervical infection, irritation, or ulceration (Niruthisard et al., 1991; Rekart, 1992; Roddy et al., 1993; Weir et al., 1995). Detergent-type spermicides alter vaginal bacteria or flora, and lead to an increase in opportunistic infections (Hooten et al., 1991; Patton et al., 1996; Rosenstein et al., 1998; Stafford et al., 1998). Such opportunistic infections are known to enhance the susceptibility of the ectocervical epithelium and the endocervical mucosa to HIV infection (Augenbraun and McCormack, 1994). Chemical irritation that disrupts the vaginal mucosa may actually enhance the risk of vaginal transmission of HIV by mucosal erosion and local inflammation (Kreiss et al., 1992; Weir et al., 1995). In a study conducted among commercial sex workers in Nairobi, in which some of the women used N-9 containing sponges, a significantly higher rate of genital ulceration and HIV seroconversion was found compared with those not using N-9 (Kreiss et al., 1992). Also, N-9 is rated as a class 2-3 toxic substance, i.e. within the toxicity range of 2.5–5% of household bleach (Gosselin et al., 1984). N-9 displays antiviral and spermicidal activities only at cytotoxic doses (D’Cruz et al., 1999a,b,c). Furthermore, recent clinical trials have shown that
vaginal contraceptive preparations containing N-9 have no effect on the transmission of AIDS and other STDs when provided as part of an overall programme to prevent heterosexual transmission (Hira et al., 1997; Roddy et al., 1998). Therefore, new, effective, safe, and female-controlled topical dual-action microbicides lacking detergent-type membrane toxicity should have clinical advantages over the currently available vaginal microbicides.

Properties required of topical microbicides

The desirable properties of a vaginally inserted viricidal spermicide are acceptability and feasibility. They must be easy to use, non-irritating, and non-toxic. Also, their efficacy must be governed by knowledge about the nature of sexual transmission of the virus. Data suggest that male-to-female transmission of HIV-1 is relatively more efficient than female-to-male transmission (Ickovics and Rodin, 1992). One study in California reported that the risk of male-to-female transmission was 17 times higher than the risk of female-to-male transmission (Ickovics and Rodin, 1992). Therefore, new, effective, safe, and female-controlled topical dual-action microbicides lacking detergent-type membrane toxicity should have clinical advantages over the currently available vaginal microbicides.

Preclinical and clinical development of microbicides

Several companies and academic groups have been working on developing clinically useful microbicides, and several microbicides are now in human clinical trials. The microbicides under development act in a variety of ways including disrupting the organism’s cell membrane or envelope, blocking the receptor–ligand interactions essential for infectivity, inhibiting the intracellular replication of the virus, altering the vaginal microenvironment, reducing the susceptibility to infection, or enhancing the local immune response.

Currently, the most popular strategy is the use of membrane-active surfactants using N-9, O-9, sodium docusate or benzalkonium chloride. Despite the known toxic effects of surfactants and the potential danger of promoting the sexual transmission of HIV, several microbicide companies are proposing the use of surfactants, especially N-9, in various new formulations or combining it with new compounds as a vaginal microbicide. Others advocate ‘well-designed clinical studies’ to improve the efficacy of N-9 (Elias and Heise, 1995; Wittkowski, 1995; Cook and Rosenberg, 1998). At least 13 of the microbicides currently being tested contain detergent-type ingredients such as N-9, O-9, benzalkonium chloride, sodium cholate or N-docosanol. It is doubtful whether inclusion of detergent spermicides in the form of film, biodhesives, acid buffer gel or liposome emulsions will improve the efficacy and safety of N-9 and other surfactants.

A second indirect approach is identification of antiviral compounds that prevent the attachment of HIV-infected lymphocytes to epithelial cells, the release of virus from these cells, or the uptake of virus by the mucosal epithelium without destroying the cell membrane (McClure et al., 1992). Polyamionic compounds (i.e. sulphated polymers such as heparin, carrageenan, dextran sulphate) prevent the binding of HIV to its host cells in vitro (Baba et al., 1988; Phillips and Pearce-Pratt, 1996) and gel formulations of these natural polymers are being evaluated for blocking or interfering effect of the receptor–ligand interactions essential for infectivity. While the sulphated polymers are potent inhibitors of HIV binding to cervical cells in vitro, they have variable efficacy as intravaginal microbicides in preclinical models. Gels containing inhibitors of intracellular or extracellular replication of viruses such as acyclic nucleoside phosphonates and organometallics are also being explored. However, none of
these microbicides have spermicidal activity. Other broad spectrum antimicrobial and spermicidal preparations in clinical trials include broad spectrum microbical preparations containing naphthalene sulphonate polymers. Yet other microbicides that are being explored tend to alter the vaginal environment (acidification of the vagina or lactobacilli fortification), reduce susceptibility (by using a combination of plantbodies, i.e. recombiant antibodies expressed in plants), or enhance the local immune response (by using immunomodulators).

The evaluation of microbicides for activity against HIV has largely depended on cell culture methods. At present, the animal models best suited for infection-blocking treatments seem to be the feline immunodeficiency virus (FIV)-infected domestic cats and the SIV/HIV-2-infected rhesus monkeys. The intravaginal effects of microbicides are primarily studied in SIV-infected monkeys where the normal disease progression is at least 10 times faster than in humans. Although the chimpanzee remains the ideal model for HIV-1 infection study (Alter et al., 1984), its utility will be limited due to their endangered species status. HIV-1 can cross the intact vaginal epithelium to establish a systemic infection in chimpanzees. Chimpanzees infected with HIV-1 do not develop AIDS but remain persistently infected. This model, therefore, allows one to test in vivo the efficacy of the test drug in the female genital tract providing data that has more predictive value regarding safety and biological activity of microbicides.

The ability of a vaginal viricide to offer protection against heterosexual or perinatal HIV transmission can only be confirmed in clinical human trials. The most rigorous and reliable approaches to measuring the efficacy of a new microbicide and obtain approval for its widespread distribution is a prospective randomized Phase III study. Such pivotal trials should include sufficient participants to have adequate statistical power to determine the clinical benefit and the frequency of any adverse effects of the experimental product. Sample size depends on the expected incidence of HIV infection in the trial participants, on the predicted reduction in incidence related to microbicide use, and other factors such as the retention and compliance of the study participants. In general, either everyone in a population at high risk of HIV infection is counselled to use both condoms and the microbicide and then groups are separated and analysed by self-reported use or everyone is counselled to use condoms and then the microbicide and a placebo are allotted randomly. Clinical trials of this kind are often conducted in developing countries among women who are at a particularly high risk for heterosexually transmitted HIV infection. At present, the major rate limiting factor is the right balance between the need to protect the rights and health of women at risk of HIV and the need to meet demands of validity and efficiency in the testing of microbicides.

Spermicidal nucleoside analogues

The life cycle of the HIV is dependent upon HIV-encoded reverse transcriptase (RT) that has been a major target for the design of potent anti-HIV agents. Nucleoside analogues, zidovudine (ZDV/AZT), didanosine (DDI), zalcitabine (DDC), stavudine (D4T), and lamivudine (3TC), and non-nucleoside analogues (nevirapine and delaviridine) which act at the RT site are the major anti-HIV drugs in clinical practice (Pauwells et al., 1990). Nucleoside analogues, such as ZDV, once phosphorylated to a triphosphate, structurally mimic one of the nucleosides and become incorporated into the elongating strand of viral DNA during the RT process; the nucleoside analogues, because they are not true nucleosides, prevent further chain linkages and thus terminate viral DNA synthesis (Mitsuya et al., 1990).

The five approved nucleoside analogue RT inhibitors belong to the broad family of 2',3'-dideoxynucleosides that comprise ZDV, DDI, DDC, D4T, and 3TC. Zidovudine was first synthesized in 1964, found to have antiretroviral activity in 1974, shown to inhibit HIV-1 in 1985, and remains the drug of choice for treatment of AIDS (Mitsuya et al., 1985). Zidovudine acts as an inhibitor of viral RT after its conversion to ZDV-5'-triphosphate by host cell kinases. The rate-limiting step for the conversion of ZDV to its bioactive metabolite, ZDV-triphosphate seems to be the conversion of the monophosphate derivative to the diphosphate derivative (McGuigan et al., 1993a). In an attempt to overcome the dependence of nucleoside analogues on intracellular nucleoside kinase activation, aryl methoxyalaninyl phosphate derivatives of ZDV (3'-azido-3'-deoxythymidine) and d4T (2',3'-dideoxy-2',3'-dideoxyhydrothymidine) have been prepared (McGuigan et al., 1993b, 1996). Such prototype drugs have improved antiviral selectivity because the dependency on the host kinases is eliminated. Secondly, the aryl phosphate derivatives have been shown to undergo intracellular hydrolysis to yield monophosphate derivatives that are further phosphorylated by thymidylate kinase to give the bioactive triphosphate derivatives in a thymidine kinase (TK)-independent fashion. However, all attempts to date to further improve the potency of the aryl phosphate derivatives of nucleoside analogues (ZDV and d4T) by various substitutions of the aryl moiety without concomitantly enhancing their cytotoxicity have failed.

ZDV is rapidly cleared from the body and its ability to cross the blood-brain barrier is less than optimal (Wang et al., 1996). The aryl phosphate derivatives of ZDV on the other hand are retained for a longer period within the body and they can inactivate HIV in cells that have low or deficient thymidine kinase activity such as monocytes and macrophages, the main carriers of HIV in semen (McGuigan et al., 1996; Quayle et al., 1997; Zhang et al., 1998). Taking advantages of this drug delivery system, with particular emphasis on nonlymphocytic cell types including spermatozoa as the infectious cells in semen,
We have synthesized novel bromo-methoxy derivatives of aryl phosphate ZDV that exhibit both potent anti-HIV and spermicidal activities.

We synthesized a panel of novel ZDV analogues and derivatives and systematically assessed their effects on human sperm function and HIV replication in HIV-1 infected peripheral blood mononuclear cells (PBMC). Exposure of the highly motile spermatozoa to ZDV, which inhibited HIV-1 replication in vitro with an IC \(_{50}\) value of 0.006 µmol/l, had no effect on sperm motility. Introduction of a bromo at the 5-position (R\(_2\)) and methoxy at the 6-position (R\(_3\)) across the double bond in the thymine ring of ZDV to yield 5-bromo-6-methoxy-5,6-dihydro ZDV (compound WHI-01) resulted in gain of significant spermicidal function (EC \(_{50}\) = 104 µmol/l) without decreasing the anti-HIV activity (Figure 1, Group A compounds). Replacement of azido group (R\(_1\)) in the pentose ring with an NH\(_2\) group (compound WHI-03) substantially enhanced spermicidal activity (EC \(_{50}\) = 12 µmol/l), but it also caused a 50-fold loss in the anti-HIV activity. The bromo-methoxy substitution was essential for the spermicidal activity of WHI-03 since 3′-amino-3′-deoxy-thymidine (compound WHI-02), which was used as a control, was not spermicidal.

Next, novel aryl phosphate derivatives of compound WHI-01 were synthesized by phosphorochloridate chemistry and examined for their anti-HIV efficacy and spermicidal activity (Figure 1, Group B compounds). Compound WHI-04 with the unsubstituted aryl moiety at the R\(_4\) position elicited three-fold greater spermicidal activity than WHI-01. Introduction of a p-methoxy (compound WHI-05, EC \(_{50}\) = 29 µmol/l), p-fluoro (compound WHI-06, EC \(_{50}\) = 15 µmol/l), or p-bromo (compound WHI-07, EC \(_{50}\) = 6 µmol/l) substituents at the R\(_4\) position in the aryl moiety further enhanced the spermicidal activity with an order of potentiation p-bromo→p-fluoro→p-methoxy. Interestingly, the p-methoxy-substituted aryl phosphate derivative WHI-05 (5-bromo-6-methoxy-5,6-dihydro-3′-azido-thymidine-5′-(p-methoxyphenyl) methoxylaminyl phosphate) had an EC \(_{50}\) value of 29 µmol/l in sperm motility assays and an IC \(_{50}\) value of 0.05 µmol/l in HIV replication assay. The lead compound, WHI-07, with a p-bromo substitution (5-bromo-6-methoxy-5,6-dihydro-3′-azido-thymidine-5′-(p-bromo ophenyl) methoxylaminyl phosphate) had an EC \(_{50}\) value of 6 µmol/l in sperm motility assays, which is consistent with a one-log higher potency that of N-9 (EC \(_{50}\) = 81 µmol/l). WHI-07 displayed a potent anti-HIV activity with an IC \(_{50}\) value of 0.005 µmol/l in HIV replication assays, which was virtually identical to that of ZDV (IC \(_{50}\) = 0.006 µmol/l) and 439-fold more potent than that of N-9 (IC \(_{50}\) = 2.2 µmol/l). The removal of the azido group of the pentose ring (WHI-11 and WHI-12) was associated with a substantial loss of the anti-HIV activity and attenuation of the spermicidal activity for these novel dual-function ZDV derivatives. The (5-bromo-6-methoxy) substituent in the thymine ring appeared to be essential for the spermicidal activity of the p-methoxy-, p-fluoro-, or p-bromo-substituted aryl phosphate derivatives of ZDV, since no spermicidal activity was observed in their absence. In contrast, the anti-HIV activity of the aryl phosphate derivatives of ZDV did not require the bromo-methoxy substitution in the thymine ring.

Ultrastuctural studies by scanning and transmission electron microscopy demonstrated that spermicidal activity of WHI-05 and WHI-07, unlike N-9, was not associated with membrane disruption (D’Cruz et al., 1998). Whereas, N-9 was cytotoxic to normal human ectocervical and endocervical cells at spermicidal doses, both WHI-05 and WHI-07 were selectively spermicidal. N-9 was spermicidal only at cytotoxic concentrations [selectivity indices (SI): 0.55 and 0.19], whereas, WHI-05 and WHI-07 showed high SI against these cells (SI: >200 and >64 for normal human ectocervical and endocervical epithelial cells respectively). These findings suggested that the spermicidal activity of WHI-05 and WHI-07 was not related to cytotoxicity.

The bromo-methoxy aryl phosphate derivatives of ZDV, which affected sperm motility, also inhibited the ability of treated spermatozoa to bind and fertilize zona-free hamster eggs (a test for human sperm competence) and sperm binding to zona-intact human eggs (D’Cruz et al., 1998). These findings clearly established that these compounds are useful in inhibiting the in-vivo fertilizing capacity of human spermatozoa once they have been exposed to these spermicidal ZDV derivatives intravaginally. Furthermore, results of our in-vivo contraceptive efficacy trials in rabbits unequivocally demonstrated that exposure of artificially inseminated semen to intravaginally applied gel-microemulsion formulation containing 2% WHI-05 or WHI-07 drastically reduced (by 81–90%) their subsequent fertilizing ability in vivo in ovulated does indicative of the inability of drug-exposed spermatozoa to reach and fertilize the ovum (O.J.D’Cruz et al., unpublished data).

In animal toxicity studies, unlike the intravaginally applied N-9, repetitive intravaginal application of WHI-07 via a cream base for 20 consecutive days did not induce any membrane disruption or an acute inflammatory response in the cervicovaginal epithelial crypts (D’Cruz et al., 1998). These features of bromo-methoxy substituted aryl phosphate derivatives of ZDV differ from those of N-9. Furthermore, intravaginal application of a gel-microemulsion formulation of 2% WHI-05 or WHI-07 for 10 days did not result in irritation or local toxicity to the vaginal epithelium or systemic absorption of these drugs in the rabbit vaginal tolerance test (D’Cruz et al., 1999a,c). We have also investigated the potential toxicity of WHI-05 and WHI-07 in two species, non-human primates and rodents. Neither WHI-05 nor WHI-07 was toxic to female cynomolgus monkeys when administered systemically as a single injection or multiple injections for 2 days via the i.v. route at doses of 20 mg/kg. Blood for haematological and clinical chemistry determinations collected from animals up to 33 days for WHI-05 and 24 days for WHI-07 showed no
Figure 1. Structure–activity relationships affecting the anti-human immunodeficiency virus (HIV) and spermicidal activity of bromo-methoxy zidovudine (ZDV) analogues and aryl phosphate derivatives of bromo-methoxy ZDV. IC50 values represent the concentration required to inhibit by 50% the activity of HIV-1 replication as measured by assays of p24 antigen production. EC50 values represent the concentration required to decrease sperm motility by 50% as measured from the concentration–response curves using a computer-assisted sperm analyser. (A) Novel ZDV analogues and derivatives; (B) novel aryl phosphate derivatives of 5-bromo-6-methoxy-5,6-dihydro ZDV (WHI-01). NA = not applicable.
significant differences. Furthermore, toxicological grading performed on post-treatment of monkeys showed no clinical evidence of significant toxicity (toxicity grade 0 to <2). In subacute i.p. toxicity studies and 13-wk intravaginal short-term toxicity studies performed in mice, no significant toxicological alterations were seen. Also, there was no evidence of teratogenicity in mice given the intravaginal application of 2% drugs via a gel–micro-emulsion base during gestation. Results of WHI-05 and WHI-07 were negative in a yeast DEL (intrachromosomal recombination resulting in deletion of intervening sequences) recombination assay and transcriptional activation of genotoxic stress-specific promoters in human hepatoma cells using the CAT-Tox(L) (chloramphenicol acetyltransferase–liver) assay.

**Spermicidal non-nucleoside inhibitors (NNIs)**

As part of our ongoing effort to find new spermicidal anti-HIV agents, we are also exploring the structure-activity relationship of novel NNIs. Non-nucleoside RT inhibitors are a structurally diverse group of compounds with a mechanism of action and binding site distinct from that of the commonly-used nucleoside analogues. The NNIs inhibit viral replication by directly binding to a specific allosteric site of HIV-1 RT near the polymerase site and interfere with reverse transcription by altering either the conformation or mobility of RT, thereby leading to a noncompetitive inhibition of the enzyme (Smerdon et al., 1994; Mai et al., 1997). The NNI binding site of HIV-1 RT is among the most extensively studied drug binding pockets. The high resolution crystal structures of HIV-1 RT from NNI-RT complexes have shown distinct properties of the NNI binding pocket within the three-dimensional structure of HIV-1 RT, which can be utilized for structure-based rational drug design (Kohlstaedt et al., 1992; Ren et al., 1995). A number of crystal structures of RT complexed with NNIs have been reported, and such structural information has provided the basis for further derivatization of NNI aimed at maximizing binding affinity for HIV-1 RT. We have constructed a ‘composite pocket’ model for the three-dimensional structure of the RT-DNA complex based on the available backbone structure of RT-DNA complex and full structure RT complexed with several NNI compounds (Vig et al., 1998ab). Structural information from several complexes was provided to a suitable working model since no experimental data regarding the crystal structure of RT–DNA–NNI complexes has been reported. We used the NNI binding site co-ordinates of nine individual RT–NNI structures to generate a composite molecular surface revealing a larger than presumed NNI binding pocket. We utilized this pocket, together with docking and a structure-based semi-empirical score function, a guide for the synthesis and analysis of novel NNIs.

Our studies on structure-based drug design by use of a computer docking procedure for the NNI binding pocket obtained from nine RT–NNI crystal structures revealed abundant sterically allowed usable space surrounding the pyridyl ring of NNIs. Therefore, we strategically designed functional groups to obtain more potent anti-HIV agents with higher affinity for NNI binding pocket of HIV RT. The computer docking procedure led to the synthesis of three novel NNIs: N-(2-(2,5-dimethoxyphenethyl))-N′-(2-(5-bromopyridyl))-thiourea (D-PBT), N-(2-(2-fluorophenethyl))-N′-(2-(5-bromopyridyl))-thiourea (F-PBT), and 5-isopropyl-2-((methylthiomythyl)(thio)-6-(benzyl)-pyrimidin-4-(1H)-one (S-DABO). All three NNIs were potent inhibitors of purified recombinant HIV RT and abrogated HIV replication in PBMCs at nanomolar concentrations (IC\textsubscript{50} < 1 nmol/l) when compared with N-9 or trovirdine (a non-nucleoside inhibitor), the most potent NNI reported to date. Also, D-PBT and F-PBT were 2–8-fold more potent than trovirdine in inhibiting recombinant HIV RT (D’Cruz et al., 1999b). The anti-HIV activity of the three novel NNIs was at least 2000-fold more potent than that of the detergent-type microbicide, N-9.

Unlike trovirdine or D-PBT, two NNIs, F-PBT and S-DABO, also exhibited concentration- and time-dependent spermicidal activity. In contrast to trovirdine or D-PBT, introduction of a fluorine atom at the ortho position of phenyl ring in PBT or an isopropyl group at the C-5 position of the thymine ring of S-DABO resulted in concentration-dependent spermicidal activity. The EC\textsubscript{50} values for the lead compound, F-PBT versus N-9 was 147 µmol/l and 81 µmol/l respectively. The spermicidal activity induced by F-PBT and S-DABO was rapid. Spermicidal activity of F-PBT and S-DABO was not accompanied by surface membrane disruption. In cytotoxicity assays, both F-PBT and S-DABO showed high SI against normal human ectocervical (SI: >6.8) and endocervical (SI: >19.8), epithelial cells respectively. These studies demonstrated that the spermicidal activity of F-PBT and S-DABO was not related to cytotoxicity. The dual anti-HIV and spermicidal activities of these novel NNIs, F-PBT and S-DABO, and lack of cytotoxicity to normal female reproductive tract epithelia, show unique clinical potential to become the active ingredients of a vaginal contraceptive for women who are at high risk for acquiring HIV by heterosexual vaginal transmission.

**Conclusions**

In a systematic effort to identify a non-detergent microbicide contraceptive potentially capable of preventing sexual transmission of HIV as well as providing fertility control, a series of novel aryl phosphate derivatives of bromo-methoxy ZDV and novel derivative of NNIs were synthesized and examined for dual anti-HIV and spermicidal activities. The potent antiviral action of the novel aryl phosphate derivatives of bromo-methoxy ZDV is particularly relevant to leukocytes in semen containing HIV because of their ability to retain full activity in seminal cells. The in-vivo antiretroviral efficacy of the lead compounds are currently being evaluated in our
laboratory using the domestic cat model for protection against vaginal and rectal inoculations of HIV and HIV-infected lymphocytes. Results of our ongoing preclinical studies demonstrated that the lead compounds, WHI-05 and WHI-07 because of their potent anti-HIV activity, in-vivo spermicidal efficacy, and lack of inflammatory and toxic effects may be useful as dual-function vaginal contraceptives for women who are at high risk for acquiring HIV/AIDS by heterosexual transmission.

Acknowledgements

These studies were supported in part by Grant HD 37357 from the National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA.

References


D’Cruz, O.J., Zhu, Z., Yiv, S.H. et al. (1999c) WHI-05, a novel bromo-methoxy substituted aryl phosphate derivative of zidovudine is a dual-action spermicide with potent anti-HIV activity. Contraception, in press.

D’Cruz, O.J., Zhu, Z., Yiv, S.H. et al. (1999c) WHI-05, a novel bromo-methoxy substituted aryl phosphate derivative of zidovudine is a dual-action spermicide with potent anti-HIV activity. Contraception, in press.


Received on March 8, 1999; accepted on August 2, 1999.