Phase 1 trial of nonoxynol-9 film among sex workers in South Africa

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Objectives: To assess the acceptability and safety of a vaginal nonoxynol-9 film in a group of sex workers at a truck stop in South Africa.

Design: A randomized double-blinded crossover trial was conducted between April 1995 and July 1995.

Intervention: Seventy-two mg nonoxynol-9 film and an identical glycerine placebo film.

Methods: Following informed consent, each study participant was randomly assigned the designated pre-coded film for 1 month. The second month was a film-free washout period and the participants used the alternate film in the third month. Besides measuring behavioural and clinical outcomes, colposcopy examination for genital lesions, serology and microbiology investigations for sexually transmitted diseases and semi-quantitative PCR for vaginal HIV load estimates were performed.

Results: Twenty women participated in the study. The women reported, on average, 19 sexual encounters per week. Vaginal intercourse was protected 25% of the time by condoms. On average, 11 vaginal films, either nonoxynol-9 or placebos were inserted per week. There were no statistically significant differences between the two treatment groups for genital lesions ($P = 0.29$), reported side effects ($P = 0.73$), and viral load ($P = 0.9$). However, the proportions of clinically detected genital lesions (six out of eight versus two out of eight) and self-reported side-effects (five out of eight versus three out of eight) were higher in the nonoxynol-9 group when compared with the placebo group. Incident sexually transmitted diseases occurred more frequently in the placebo group. An increased viral load was associated with the development of a genital lesion (relative risk, 6.0; 95% confidence interval, 0.81–44.4).

Conclusions: The 72 mg film formulation of nonoxynol-9 was an acceptable product for use in this population of sex workers. Although no statistically significant differences in adverse outcomes were detected, clinically there appeared to be an increase in minor lesions and self-reported side-effects with nonoxynol-9 and less protection against sexually transmitted diseases with the placebo. Furthermore, HIV shedding was correlated with the presence of incident vaginal or cervical lesions. This brings into question the potential narrow margin of safety for this product; additional Phase 2 studies are therefore required.

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Keywords: nonoxynol-9 film, microbicide, sex workers, genital lesions, vaginal HIV load, douching, intravaginal substance use

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Introduction

South Africa is experiencing one of the fastest growing AIDS epidemics in the world. The annual, anonymous, HIV seroprevalence surveys amongst ante-natal clinic attendees has demonstrated a rise in infection from 0.76% in 1990 to 14.9% in 1996 [1]. A number of factors have influenced the rapid spread of HIV infection in South Africa, such as high background levels of other sexually transmitted diseases (STD) [2,3], the migrant labour system with its concomitant oscillatory migration and related sexual networking patterns [4], limited access to services, and the limited ability and or lack of desirability to adopt safer sex practices [5]. Data for women sex workers show that more than 50% are infected with HIV [6]. In the face of this rapidly progressing epidemic, prevention strategies, targeted at women, have lagged behind [7].

We have reported previously [8] on the social context of sex work at a long distance truck-stop in South Africa and in particular on the repercussions for the group of sex workers who insisted on condom use in terms of loss of income, loss of clients, and experience of violence. The impetus for this study came from this group of sex workers who recognized their risk of acquiring HIV infection as well as their limited ability to get their client's and other sexual partners to consistently use male condoms. This identified an urgent need to increase prevention options for women, under their control.

Nonoxynol-9 was the only potential intravaginal microbicide available for study at the time. Although the use of nonoxynol-9 as a contraceptive has been established [9], its role as a microbicide is more controversial [10]. Notwithstanding variability in study populations, dose of nonoxynol-9, choice of placebo and vehicle of delivery of the microbicide, a protective effect of nonoxynol-9 has been demonstrated against certain bacterial STD [11]. Contentious issues focus on the efficacy of nonoxynol-9 against HIV, adverse reactions, and dose-related effects [12].

The implications of frequent use of a low dose film for a higher frequency of coital acts under conditions of actual use had not been addressed. Further, the practice of frequent douching and extensive intravaginal substance use common in our study population necessitated as assessment of the safety and acceptability of the nonoxynol-9 film in our population before proceeding to an efficacy trial.

Methods

Approval for this study was obtained from the Ethics Committee of the Faculty of Medicine, University of Natal.

Study population

All female sex workers based at a popular truck stop mid-way between Durban and Johannesburg were invited to participate in this study. HIV infection was not an exclusion criterion. Of the 21 sex workers at this stop only one women was excluded because she was pregnant. Eligibility criteria included women over the age of 18 years, being present at the truck-stop for more than 3 months and with the intention to remain at the truck-stop for the next 3 months, and a written declaration of a willingness to participate in the trial with full knowledge of the experimental nature of the endeavour.

Study design

To assess the safety and acceptability of nonoxynol-9, a randomized double-blinded crossover trial was conducted between April and June 1995 in KwaZulu-Natal, South Africa. A 72 mg nonoxynol-9 film and an identical glycerine containing film in matching packaging was used in the intervention and placebo arms of the trial, respectively. The intervention and placebo film could only be differentiated by an independent statistician from the serial numbers on the packaging. On enrolment each study participant was allocated a study number. Each participant was allocated film based on her study number and the corresponding serial number allocated by the statistician. Each participant was provided with the designated. pre-coded film for 1 month. The second month was a film-free washout period. During the third month each participant who was assigned to the placebo arm in the first month of the trial was now assigned to the intervention arm of the trial and vice versa.

Based on previous data on coital frequency per month [8] each participant was allocated 40 films on a 2-weekly basis and informed that if they required more film this would be made available on request.

At the enrolment visit each participant was instructed on maintenance of a pictorial coital log and on product use. The first dose of product was inserted at the clinical facility under observation. The women were instructed to insert the film daily at least 10 min before coitus.

At enrolment a behavioural questionnaire was administered. This included a recording of douching and intravaginal substance use, sexual behaviour, gynaecological history, and contraceptive history on a standardized, pre-piloted questionnaire.

Follow-up visits were conducted on a 2-weekly basis. At enrolment and each of the fortnightly visits clinical and acceptability assessments were completed. Each visit included the following analyses. (i) Monitoring of product usage. The number of unused films during the previous 2 weeks was compared to the number of films

supplied and the number of films used, coital frequency, and condom use that was recorded on the coital log. The unique identifier on the returned product was compared to that on the allocated product for each participant. (ii) Clinical examination and colposcopy of the vulva, vagina and cervix was undertaken. Ulcerations, abrasions, ecchymoses, petechiae, oedema, and erythema was documented in accordance with the World Health Organisation guidelines for colposcopy assessments [13]. (iii) Appropriate specimens were collected for microbiological testing as follows: 'first catch' urine specimens were taken and stored at –22°C before being transported to the South African Institute for Medical Research Laboratories in Johannesburg, South Africa for determination of *Neisseria gonorrhoea* and *Chlamydia trachomatis* infection using the ligase chain reaction (Abbott Laboratories, Chicago, Illinois, USA). Venous blood was obtained and tested for syphilis and HIV infection. Syphilis serology comprised the rapid plasma reagin test (Brewer Diagnostic Kits, BBL Microbiological Systems, Cockeysville, Maryland, USA). A rising rapid plasma reagin titre on follow-up examination with or without a clinically detected lesion was regarded as an indicator of active syphilis. In addition, specimens were collected from the base of any detected ulcers and cultured for *Haemophilis ducreyi* and microscopically examined (dark field) for the presence of *Treponema pallidum*. (iv) Virological assays were conducted for the detection of antibodies to HIV using a recombinant HIV1/HIV2 enzyme-linked immunosorbent assay test (Abbott Laboratories); all reactive tests were confirmed by Western blotting (Diagnostic Biotechnology, Singapore, Singapore). Vaginal viral load assessments were conducted on all participants including HIV-negative individuals who served as controls for the possibility of detecting HIV deposited in the vagina during coitus. The first procedure during the vaginal examination was the collection of a saline vaginal wash of 4 ml aliquoted in two vials of equal volume. Specimens were stored at –20°C until testing. A total of 140 vaginal/cervicovaginal washes were collected from participants in both arms of the trials, however only 83 (59.2%) of these specimens were available for HIV RNA detection due to losses from storage or transport. All testing was done at the Institute of Tropical Medicine (Antwerp, Belgium) using the Institute of Tropical Medicine protocol for the semi-quantification of HIV RNA in vaginal fluid (Perkin Elmer, Antwerp, Belgium). Using this method a maximum of 8.8 copies/1.6 ml of washing fluid was detected. (v) Product acceptability was assessed using a questionnaire which included the reporting of side-effects, douching and intravaginal substance use and partners'/clients' reactions.

All participants were reimbursed for transport costs and were provided with refreshments during each visit to the clinic in Durban. In addition, at each visit, each participant was vigorously counselled on safer sex practices, supplied with male condoms and treated for STD where indicated.

Data analysis

The main outcomes of interest were the number of women with: (i) lower genital tract symptoms; (ii) new genital lesions; (iii) laboratory diagnosed STD; and (iv) the presence of vaginal HIV RNA. Acceptability outcomes recorded as open-ended questions were coded for content and treated as categorical variables. An intention-to-treat analysis was performed. The total proportions of adverse outcomes were ascertained. The proportion of individuals with discordant adverse outcomes among these individuals were compared in the two treatment groups, using a binomial exact test because of small expected frequencies. All *P*-values were two-tailed and a P-value < 0.05 was regarded as statistically significant.

Results

A total of 20 female sex workers were enrolled into this study, 19 of whom each contributed 12 weeks of follow-up. One participant left the truck stop during the fourth week of the washout period, hence a slightly shorter cumulative observation period in the nonoxynol-9 arm of the trial. At enrolment, the participants in the study and placebo arm of the trial were comparable in terms of socio-demographic characteristics, frequency of coitus, douching or intravaginal substance use, prevalence of STD and colposcopy assessments (Table 1). The HIV seroprevalence among participants was 70% (14) at enrolment. There were no HIV seroconversions during the trial.

Exposure to product and adherence to instructions on product use

An analysis of the coital log data demonstrated no significant differences in either condom use or film use

Table 1. Baseline characteristics of study population after randomization.

Characteristics	Group 1 (n = 10)	Group 2 (n = 10)
Mean age [years (range)]	28 (20–38)	28 (19–43)
Number of clients per week [n (range)]	8 (7–20)	10 (6–20)
Coital acts per week [n (range)]	20 (5–30)	22 (8–30)
Intravaginal substance use (yes)	8	8
Vaginal discharge (yes)	8	4
Sexually transmitted diseases detected		
HIV	7	7
Syphilis	5	6
Gonorrhoea	2	2
Chlamydia	0	1
Abnormal colposcopy		
Erythema	3	4
Petechiae	1	1

Table 2. Assessment of exposure to intervention and compliance.

Weekly coital log records (n = 20)	Placebo film (n)	Nonoxynol-9 film (n)
Films used (mean)	12	10
Condoms used (mean)	5	4
Coital acts for which neither films nor condoms were used used (mean per week)	2	2
Total coital acts (mean per week)	19	16

between each arm of the trial (Table 2). The unique identifiers on the used and unused packages corresponded to that allocated to the individual participant in every instance in both arms of the trial. A total of seven individuals returned incomplete coital logs and reported problems completing the logs related to the lack of time, failure to recall or misplaced sheets. This was not discordant for the nonoxynol-9 or placebo arm.

Acceptability of product

Of the eight women who reported side-effects, the proportion reporting side-effects in the nonoxynol-9 group (five out of eight) was not significantly different ($P = 0.73$) from the proportion reporting side-effects in the placebo group (three out of eight) (Table 3). The most common complaint was a vaginal itch (four women). Other complaints included vaginal burning (two women) and pain during coitus (two women). The remaining participants (12) reported a positive experience when using the film. Common comments made by participants on describing desirable outcomes of film use included: 'feeling clean and fresh', 'tasting and smelling good', and 'feeling tight and dry'.

No participant reported difficulty in inserting the product. Product use was consistently high throughout the study period (2–5 times per day in both arms of the trial). None of the participants informed their clients that they were using a film, or did any client spontaneously indicate awareness that the participant was using a film. Half of the participants in both arms of the study informed their regular partner about the use of film and experienced no problems with this disclosure. All participants who reported a douching practice or intravaginal substance use at baseline continued to do so during the progress of the trial. No other differences between film use with the regular partner versus clients were reported.

Safety of product

Genital lesions

Nine individuals with genital lesions were detected (six had a lesion in the nonoxynol-9 arm only; two in the placebo arm only; and one in both arms). Erythematous lesions were detected in the one woman with concordant lesions in the two arms of the trial. Five erythematous lesions and one petechial haemorrhage occurred in the women in the nonoxynol-9 arm compared with one erythematous lesion and one abrasion in the placebo arm. This was the only incidence of epithelial disruption in the trial. The higher proportion of lesions in the nonoxynol-9 arm (six out of eight) was not statistically significant when compared to the proportion of lesions in the placebo arm (two out of eight), ($P = 0.29$; Table3).

Effect on STD

Of the four women with discordant incidence of STD, gonorrhoea and chlamydia infection were detected in three women from the placebo arm and *Haemophilis ducreyi* was cultured from the base of a vulval ulcer in one woman in the nonoxynol-9 arm of the trial (Table 3). The difference in the proportion of STD in each of the trial arms was not statistically significant ($P = 0.62$). In one woman, infection with *N. gonorrhoea* was detected in both arms of the trial, after having received appropriate treatment and having been infection free in the washout period.

HIV RNA was detected in the cervico-vaginal washings of five out of the 14 HIV-positive women. Of these, two occurred in the nonoxynol-9 arm compared with one in the placebo arm ($P = 0.9$) (Table 3). Of the remaining positive results, one occurred in the washout period of an individual, whereas the other occurred in the same individual with a positive result in each arm of the trial. On development of a genital lesion during the study period 50% (four out of eight) had a positive HIV RNA whereas HIV RNA was positive in only 8.3% (one out of 12) of women without genital lesions (relative risk, 6.0; 95% CI, 0.81–44.4). No viral RNA was detected in any of the 36 specimens from the six HIV-negative participants.

Table 3. Comparison of treatment effects.

Adverse outcome	Individuals with concordant adverse outcomes	Individuals with discordant outcomes under		*P*
		Nonoxynol-9	Placebo	
Genital lesions	1	6	2	0.29
Side-effects	0	5	3	0.73
Sexually transmitted diseases	1	1	3	0.62
Viral load	1	2	1	0.9

Discussion

The use of the 72 mg film was found to be acceptable in this group of sex workers whose preference is for dry sex which they achieve through frequent douching and extensive intravaginal substance use. Whilst some participants experienced problems with keeping records on coital logs, the trend was that of consistently high product use throughout the study period.

In our study the product was used several times in conjunction with sex work, post-coital douching practices, high prevalence of STD and a high frequency of coital acts; the incidence and severity of the genital lesions described here are comparable to those found in a recent study [14] of a very low dose (52 mg) bio-adhesive gel formulation of nonoxynol-9 used once daily. For these reasons, the clinical findings of an increase in minor (erythematous) genital lesions and self-reported side-effects under nonoxynol-9, together with an increased viral burden associated with even minor genital lesions becomes worrisome because it brings to question the potential narrow margin of safety for this product.

Coital logs coupled with the return of used packets were used as indication of exposure and condom use. The extent to which these tools reflect a 'social desirability' response is difficult to predict. A reliance on self-reported sexual behaviours as a measure of exposure requires further consideration in the planning of efficacy trials.

It is generally not recommended for a safety trial to be conducted under high-risk conditions because apart from the risk to the participants control of various confounders is often inadequate because of the small sample requirements of phase 1 trials. For these reasons the crossover design and analyses proved to be of benefit, where each individual served as her own control. The washout period of 1 month was long enough to prevent the possibility of carry-over effects.

Although the use of nonoxynol-9 did not increase the signs and symptoms of genital lesions to statistical significance when compared with the placebo film, the clinical findings were of concern. It was decided that the new formulation of nonoxynol-9, a low dose (52 mg), long-acting bio-adhesive gel, might prove to be the better option. Following this safety trial the research endeavour was expanded to include several truck stops in this region. Activities in the group included ongoing counselling, an adult literacy programme and the initiation of an UNAIDS funded efficacy trial of the nonoxynol-9 gel.

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References

1. Abdool Karim Q, Abdool Karim SS. **South Africa: Host to a new and emerging HIV epidemic.** *Sex Transm Infect* 1999, **75**:139–147.
2. Abdool Karim SS. **Challenges to the control of sexually transmitted diseases in Africa (editorial).** *Am J Public Health* 1994, **84**:1891–1893.
3. Pham-Kanter GBT, Steinberg MH, Ballard RC. **Sexually transmitted diseases in South Africa.** *Genitourin Med* 1996, **72**:160–171.
4. Jochelson K, Mothibeli M, Leger JP. **Human immunodeficiency virus and migrant labour in South Africa.** *Int J Health Serv* 1991, **21**:157–173.
5. Abdool Karim Q, Tarantola D, As Sy E, Moodie R. **Government responses to HIV/AIDS in Africa: what have we learnt?** *AIDS* 1997, **11** (suppl B):S143–S149.
6. Ramjee G, Abdool Karim SS, Sturm AW. **Sexually transmitted infections among sex workers in KwaZulu Natal, South Africa.** *Sexually Trans Diseases* 1998, **25**:346–349.
7. Ulin P. **African women and AIDS: negotiating behavioural change.** *Soc Sci Med* 1992, **34**:63–73.
8. Abdool Karim Q, Abdool Karim SS, Soldam K. **Reducing the risk of HIV infection among South African sex workers: socio-economic and gender Barriers.** *Am J Public Health* 1995, **85**:1521–1525.
9. Department of Health and Human Services, FDA. Federal Register 1980, **45**:822014–82049.
10. World Health Organisation/Global Programme on AIDS. *Report on a meeting on the development of vaginal microbicides for the prevention of heterosexual transmission of HIV*. Geneva, November 1993.
11. Feldblum PJ, Bernadik E, Rosenberg MJ. **Spermicide use and sexually transmitted disease.** *JAMA* 1988, **259**:2851–2857.
12. Elias CJ, Heise LL. **Challenges for the development of female-controlled vaginal microbicides.** *AIDS* 1994, **8**:1–9.
13. WHO/GPA/RID/CRD. *Manual for the Standardisation of Colposcopy for the Evaluation of Vaginally Administered Products.* Geneva: World Health Organisation; 1995.
14. Van Damme L, Niruthisard S, Atisook R, *et al.* **Safety evaluation of nonoxynol 9 gel in women at low risk for HIV infection.** *AIDS* 1998, **12**:433–437.