

Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial

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Summary

Background Nonoxynol-9 (rINN, nonoxinol-9) is an over-the-counter spermicide that has in-vitro anti-HIV-1 activity. Results of studies of its effectiveness in prevention of HIV-1 infection in women have been inconclusive. We aimed to assess effectiveness of this vaginal gel.

Methods We did a randomised, placebo-controlled, triple-blinded, phase 2/3 trial with COL-1492, a nonoxynol-9 vaginal gel, in 892 female sex workers in four countries: Benin, Côte d'Ivoire, South Africa, and Thailand. 449 women were randomly allocated nonoxynol-9 and 443 placebo. Primary endpoint was incident HIV-1 infection. Secondary endpoints included *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections. Analysis was by intention to treat.

Findings 765 women were included in the primary analysis. HIV-1 frequency in nonoxynol-9 users was 59 (16%) of 376 compared with 104 (27%) of 389 in placebo users (402.5 vs 435.0 woman-years; hazard ratio adjusted for centre 1.5; 95% CI 1.0–2.2; $p=0.047$). 239 (32%) women reported use of a mean of more than 3.5 applicators per working day, and in these women, risk of HIV-1 infection in nonoxynol-9 users was almost twice that in placebo users (hazard ratio 1.8; 95% CI 1.0–3.2). 516 (68%) women used the gel less frequently than 3.5 times a day, and in these, risk did not differ between the two treatments. No significant effect of nonoxynol-9 on *N gonorrhoeae* (1.2; 0.9–1.6) or *C trachomatis* (1.2; 0.8–1.6) infections was reported.

Interpretation This study did not show a protective effect of COL-1492 on HIV-1 transmission in high-risk women. Multiple use of nonoxynol-9 could cause toxic effects enhancing HIV-1 infection. This drug can no longer be deemed a potential HIV-1-prevention method. Assessment of other microbicides should continue.

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Introduction

Although the male condom, when used consistently and correctly, provides high levels of protection against HIV-1 and other sexually transmitted infections, negotiating its use is not always feasible for many women. Therefore, there is need for a female-controlled method for prevention of HIV-1. Research on microbicides is part of this global effort.

The product that has been tested most is the spermicide nonoxynol-9 (recommended international name nonoxinol-9), which shows in-vitro activity against HIV-1 and other sexually transmitted infections^{1–5} and can prevent simian immunodeficiency virus infection in macaques.⁶ Results of several studies in women showed that nonoxynol-9 had a protective effect against *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection.^{7–10}

At the time we planned our study, data for HIV-1 prevention were conflicting. Although results of an observational study in female sex workers in Cameroon showed a protective effect in more consistent spermicide users than less consistent users (relative risk 0.1; 95% CI 0.1–0.6),¹¹ results of a randomised placebo-controlled trial did not show a significant protective effect (1.6; 0.8–2.8).¹² The high number of toxic effects seen in this study was thought to be attributable to the high dose of nonoxynol-9—ie, 1000 mg, with about 50% of this dose bioavailable.

Because nonoxynol-9 is readily available, low in price, and has been on the US market as an over-the-counter product since the 1960s, we thought controversy surrounding the drug as a potential HIV-1-prevention method needed to be resolved. We decided to study a new gel formulation with a low nonoxynol-9 dose (52.5 mg), COL-1492, which covers the cervix and the vaginal walls and gives immediate availability of the drug. Because of the known dose-dependent effect of this drug,¹³ the absence of any local toxic effects of the gel was documented first in women in developed countries, then by a study of the phase 3 target population.^{14,15} Results of both trials showed no difference between nonoxynol-9 and placebo with respect to frequency of lesions.

Our aim was to compare the effectiveness of nonoxynol-9 with placebo gel in prevention of HIV-1 infection in HIV-1-negative female sex workers.

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Participants and methods

Participants

Between September, 1996, and June, 2000, we did a randomised, placebo-controlled, triple-blind trial, in which we screened healthy, HIV-1-negative female sex workers in South Africa (Durban and Johannesburg), Thailand (Bangkok and Hat Yai), Benin (Cotonou), and Côte d'Ivoire (Abidjan) for inclusion in our study. Women were recruited from clinics for sexually transmitted infection in Bangkok, Hat Yai, and Johannesburg; from clinics for female sex workers in Cotonou and Abidjan; and in Durban through the Medical Research Council, which has close links with sex workers at truck stops along the main road between Durban and Johannesburg.

We used the following criteria for enrolment: age 18 years or older in Benin, Côte d'Ivoire, and Thailand, or 16 years or older in South Africa; willingness and ability to give informed consent and to adhere to the study protocol; not users of intravenous drugs or intravaginal spermicides other than the study drug; not pregnant or no wish to become pregnant in the next 6 months; and not allergic to latex or a study-gel ingredient.

The study was approved by all local institutional and national ethics review committees in South Africa, Thailand, Benin, and Côte d'Ivoire, and by the ethics review committees of UNAIDS, Centre Hospitalier Affilié Universitaire de Québec (Quebec, Canada), the US Centers for Disease Control and Prevention (CDC, Atlanta, USA), and the Institute of Tropical Medicine (ITM, Antwerp, Belgium).

Procedures

At screening we obtained verbal consent and gathered sociodemographic characteristics and data on sexual behaviour, including methods used for contraception and prevention of sexually transmitted infections. A gynaecological examination and urine pregnancy test were done. We took a swab from the posterior fornix for diagnosis of candidiasis and *Trichomonas vaginalis* by wet-mount microscopy. We took three endocervical swabs: one for *N gonorrhoeae* culture; one for diagnosis of *C trachomatis*; and one for storage and for further testing at ITM. Blood was drawn for HIV-1 and syphilis serological testing. In Cotonou, we used a fingerprick to obtain blood for HIV-1 testing. Details of laboratory methods are given on *The Lancet's* website (<http://image.thelancet.com/extras/02art3365webmethods.pdf>). All women received HIV-1 pre-test counselling and safer-sex messages. If a curable sexually transmitted infection was diagnosed, we gave treatment in accordance with local guidelines. We asked women to return to the centre within 28 days for study enrolment.

At enrolment, comprehensive study information was given to every participant, and we obtained written informed consent. If the woman fulfilled all criteria for enrolment, she was randomly allocated to one of the treatment groups (see below for randomisation procedure). All women received post-test HIV-1 counselling. We did a gynaecological examination and urinary pregnancy test (see screening visit) and took venous blood for HIV-1 and syphilis tests. Again, women received HIV-1 pre-test counselling, safer-sex messages, and treatment for sexually transmitted infections if needed. We asked women to return to the clinic every month for a follow-up visit.

At every follow-up visit, we asked questions about any difficulties that might have arisen, and also about sexual behaviour, including gel and condom use, and the gel's acceptability. We did a gynaecological examination (see screening visit) and took a blood sample for HIV-1 and syphilis serological testing. In Abidjan and Cotonou, we

took venous blood at enrolment and then every 6 months; between these visits, we took blood by fingerprick every 2 months. Whenever a curable sexually transmitted infection was diagnosed, treatment was given free of charge. Women who seroconverted received appropriate counselling. HIV-1 pre-test messages and advice on male condom use were given at every visit. We assessed the participants' understanding of the study procedures on a regular basis.

Women received a supply of study gel (nonoxynol-9 or placebo) and of male condoms to fulfil their needs until the next visit. They were asked to use the male condom for every sexual act and to apply the gel for vaginal intercourse if they had cleaned their vagina after last intercourse. There was no set limit on the number of gel doses that could be used per day.

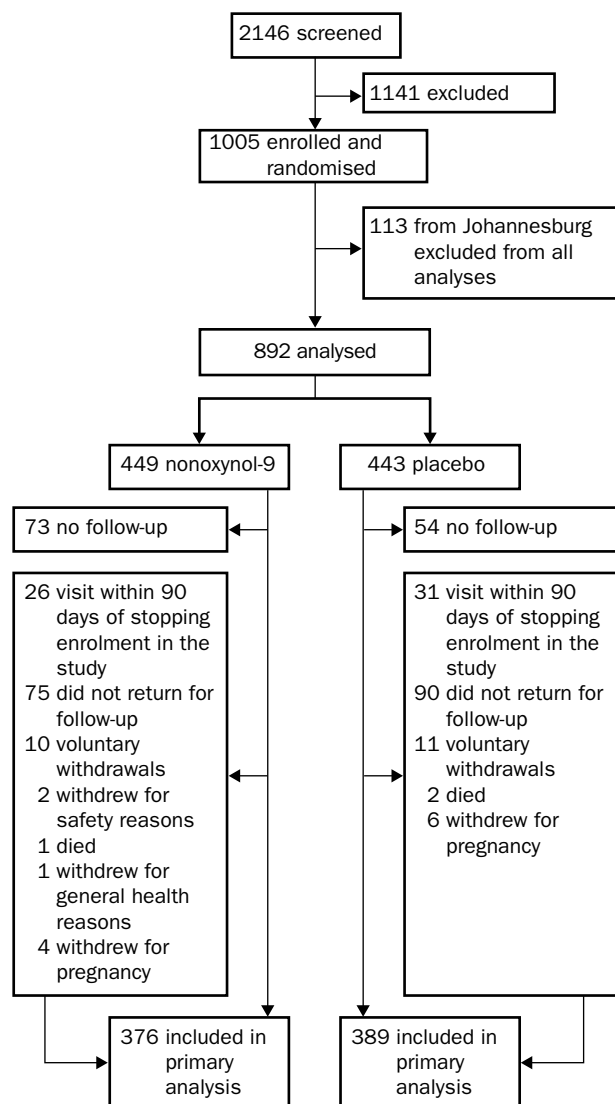
COL-1492 gel (Columbia Laboratories, New York, NY, USA) contains 3.5% (52.5 mg) nonoxynol-9; other constituents include carbomer, a polymer with bioadhesive properties. The placebo gel, a vaginal moisturiser marketed as Replens (polycarbophil; Columbia Laboratories, Paris, France), was the same as COL-1492 but did not contain nonoxynol-9 and had more carbomer. The nonoxynol-9 and placebo gels had slightly different pH ranges. Both gels were packaged in single-dose, disposable plastic applicators designed to deliver 1.5 g gel, and were supplied by Columbia Laboratories (Paris, France). We also provided male latex condoms, not lubricated with spermicides.

Randomisation was done per centre, by computer in blocks of 16. There were eight allocation groups (A to H), four groups were randomly allocated placebo and four nonoxynol-9. Centres were provided with sealed envelopes—one per participant—containing a piece of paper showing to which group (A to H) the woman was to be allocated. Envelopes were prepared by Columbia Laboratories, and had the participant number printed on the outside. Participant numbers were assigned consecutively. Participants, investigators, data managers, statisticians, and trial coordinators were masked to treatment allocation.

Our study was set up as a phase 2/3 trial. Women who were enrolled in the introductory phase 2 could continue their participation without discontinuation or interruption of treatment, and were followed-up until the start of phase 3 (Durban only). Results of colposcopy examinations done during phase 2 have been presented elsewhere.¹⁵ 320 women were included in that analysis.

The phase 3 part of the study, in which colposcopy was not done, started after a data safety and monitoring board decision in August, 1997. The Bangkok centre participated in the phase 2 study only, because of the low incidence of HIV-1 in their sex-worker population. Similarly, enrolment in Hat Yai was switched to another clinic for the phase 3 study because of the low HIV-1 incidence in the first clinic. The centre in Johannesburg was closed because of a noted retention rate of 29% after 6 months, which compromised quality and interpretation of data. Data from this centre were thus excluded from all analyses.

In the introductory phase 2, we used a simple monthly pictorial coital log chart, on which we asked women to record the type of each sexual act (vaginal, oral, anal), if they had used the gel and the condom, and if they had cleaned their vagina. Later, at the start of phase 3, we used a more complex monthly coital log chart, on which women were asked to record the type of each sexual act (vaginal, oral, anal), the type of partner (client or regular partner), use of condom and gel, if any condom difficulty (ie, breaking or slipping) had occurred, and if they had washed their vagina. We changed this procedure a second time in



Trial profile

November, 1998, because we questioned the value of the recorded data because we saw women completing their booklets while waiting at the clinic. From then on, this information was obtained by interviewing of women about their last working day for clients and the last week for regular partners.

The primary endpoint of the study was incident HIV-1 infection. Secondary objectives included the effectiveness of this drug in prevention of chlamydial infection, gonorrhoea, trichomoniasis, and genital ulcer disease; and safety and acceptability of the gel under situations of long-term use.

Incident HIV-1 infections were confirmed at the reference laboratory (ITM). Time of seroconversion was defined as the midpoint between the last HIV-1-negative test and the first HIV-1-positive test confirmed at ITM.

For *N gonorrhoeae* and *C trachomatis* infections, we analysed the first episode only. A woman was judged positive for *N gonorrhoeae* when she had a positive culture at the centre or when both PCR tests at ITM were positive. We deemed her positive for *C trachomatis* if she had a positive enzyme immunoassay test confirmed by a blocking assay or a positive PCR test at ITM. If there was a discrepancy between results reported in the case-record form and in the local laboratory file, we judged ITM result the correct one. In cases of inconsistency for which no swab

	Nonoxynol-9 (n=376)	Placebo (n=389)
Age (years, median [IQR])*	26.0 (22–31)	26.0 (22–31)
Clients per day (median [IQR])†	3 (2–5)	3 (2–5)
Years of education (median [IQR])	6 (4.0–9.5)	6 (4.0–9.0)
Condom used for ≥50% of sexual acts		
With clients‡	253 (67%)	256 (66%)
With partners	27/225 (12%)	14/230 (6%)
Routine washing of the vagina done		
Number of times per day (median [IQR])§	264/264 (100%) 3.0 (2.0–3.0)	277/277 (100%) 3.0 (2.0–3.0)
Anal sex		
Ever	52 (14%)	56 (14%)
Frequency per week (median [IQR])¶	2.0 (2.0–4.0)	2.0 (1.0–3.0)
Abnormal vaginal discharge **		
	51 (14%)	63 (16%)
Clinical evidence of cervicitis**		
	31 (8%)	39 (10%)
Infection		
<i>Trichomonas vaginalis</i> **	14 (4%)	13 (3%)
<i>Candida albicans</i> **	55 (15%)	55 (14%)
Syphilis**	39 (10%)	47 (12%)
<i>Chlamydia trachomatis</i> **	18 (5%)	16 (4%)
<i>Neisseria gonorrhoeae</i> **	17 (5%)	22 (6%)

Data are number (%) unless otherwise stated. *Data for one participant missing in nonoxynol-9 group. †Data for one participant missing in placebo group. ‡Data for three participants missing in placebo group. §Nonoxynol-9, n=255; placebo, 269. ¶Nonoxynol-9, n=51; placebo, 55. ||Seen at clinical examination. **Seen at admission.

Table 1: Baseline characteristics of women with at least one follow-up visit

was available at the ITM, we recorded the result as unknown.

We classified lesions in the genital area—ie, cervix, vagina, vulva—noted on clinical examination (naked eye), as being with or without epithelial disruption, having unknown epithelial disruption, or as ulcerations for which localisation was not specified. We included all lesions, irrespective of their possible cause.

We judged women who were in active follow-up—ie, they had a visit in the previous 6 weeks—on the date enrolment in the study stopped, and who did not return within 45 days for a closeout visit, to have completed the trial, because participants were reluctant to return to the clinic once they heard that the gel was no longer being distributed.

Statistical analysis

We assumed a 5% annual HIV-1 incidence, thus we needed 2500 woman-years to detect a 50% reduction in risk with 90% power, a two-sided test at a significance level of 5%, one to one random allocation, and 60% retention per year. We needed 100 endpoints to meet these assumptions.

We planned an interim analysis at every 25 HIV-1-seroconversions on the basis of the masked randomisation codes. We presented the results of these interim analyses to an independent data safety and monitoring board. In the event of a significant result ($p < 0.001$), the randomisation code would have been broken. The data safety and monitoring board decided to stop the trial when the required 100 events had arisen.

The main analyses were by intention to treat, judged time to the first event, and were stratified by centre. We used medians and proportions to compare baseline characteristics of the two treatment groups. Time to first event was analysed with log-rank tests for differences between treatment groups and Kaplan-Meier estimates for survival. Hazard ratios were calculated with Cox proportional-hazards regression models.

	Nonoxynol-9				Placebo				Hazard ratio (95% CI)	p
	n	Woman-years	Number of seroconversions	HIV-1 incidence per 100 woman-years	n	Woman-years	Number of seroconversions	HIV-1 incidence per 100 woman-years		
Centre										
Abidjan	78	67.9	0	0	84	67.3	5	7.4	0 (0.0-0.8)*	0.03
Bangkok	26	5.8	0	0	25	5.3	0	0	NA	..
Cotonou	100	107.2	12	11.2	103	121.1	10	8.3	1.4 (0.6-3.1)	0.39
Durban	94	162.1	42	25.9	93	182.3	30	16.5	1.6 (1.0-2.5)	0.06
Hat Yai phase 2	22	6.8	0	0	25	9.0	0	0	NA	..
Hat Yai phase 3	56	52.7	5	9.5	59	50.0	0	0	∞ (1.2-∞)*	0.03
All centres	376	402.5	59	14.7	389	435.0	45	10.3	1.5 (1.0-2.2)†	0.047

NA=not available. *Exact 95% CI. †Adjusted for centre.

Table 2: HIV-1 incidence per treatment group per centre, and overall

The population included in the primary analysis comprised all HIV-1-negative women enrolled in the trial who had at least one HIV-1 test during follow-up. Genital lesions at baseline were not counted as incident lesions. Women in the primary analysis were included in the analysis of gonorrhoea or chlamydia incidence if they did not have the corresponding infection at baseline.

We did exploratory analyses of HIV-1 and genital lesion endpoints with the Cox proportional-hazards model for time-dependent covariates to investigate the effect of different measures of exposure derived from the coital log books.

Role of the funding source

UNAIDS was the main sponsor of the trial and had an input in protocol design and in submission of the article. Columbia Laboratories provided the study product and did randomisation.

Results

2146 women were screened and 1005 were enrolled. A total of 892 women (excluding 113 from Johannesburg) were randomly allocated (figure), of whom 127 had no HIV-1 test after enrolment. These women were excluded from the primary analysis, which thus included 765 women. 563 women completed the study, including 57 women with a follow-up visit within 6 weeks before the end of study enrolment. 37 women withdrew during the study (figure).

The overall retention rate of participants in the study was 71% after 24 weeks and 68% after 48 weeks, which is closely similar to rates assumed for sample-size calculations. The retention rate differed substantially between centres, ranging from 58% at 48 weeks in Cotonou to 88% in Durban (data for individual centres available from authors). The rates by treatment at 24 weeks were 69% and 73% in the nonoxynol-9 and placebo groups, respectively, and at 48 weeks, 65% and 70%, respectively.

Both treatment groups were balanced with respect to baseline characteristics (table 1). However, differences were noted between centres. Women in Durban were on average younger than women in Bangkok (24 vs 29 years). Condom use with clients at baseline differed substantially, with all women in Bangkok (n=51) reporting 100% condom use and only 31 (17%) of 187 women in Durban reporting that they were protected by condoms for more

than 50% of sex acts. At baseline, anal sex was reported by 76 (41%) of 187 women in Durban, 17 (8%) of 203 in Cotonou, and fewer than 5% in Abidjan and Hat Yai.

When looking at the number of vaginal sex acts with clients, in 19 225 (9%) of 201 585 acts in the nonoxynol-9 group, the gel was used without a condom, in 35 825 (17%) the condom was used without the gel, and in 146 536 (70%) condom and gel were both used. For the placebo group, these numbers were 22 829 (9%), 45 826 (18%), and 186 348 (72%) of 255 003, respectively.

199 (53%) and 202 (52%) women in the nonoxynol-9 and placebo groups, respectively, reported use of the gel in 95% or more vaginal acts with clients, and 128 (34%) and 121 (31%), respectively, reported use of the gel for all their vaginal sex acts with clients. Median gel use per working day was 2.2 applicators (IQR 1.1-4.0) in the nonoxynol group and 2.4 (1.0-3.9) in the placebo group.

104 seroconversions arose during follow-up, of which 59 were in the nonoxynol-9 group (table 2). A higher incidence of HIV-1 in the nonoxynol-9 group than in the placebo group was noted in three of the four centres in the phase 3 study. In only one centre (Abidjan) was the reported HIV-1 incidence lower in the nonoxynol-9 group than in the placebo group (treatment by centre interaction, p=0.003). In one centre (Cotonou) the incidence of HIV-1 infection in the nonoxynol-9 group was higher than in the placebo group in the first year of follow-up but was lower than placebo in the second year. The non-proportionality of risks was significant in this centre (p=0.04), but was not seen in other centres nor in the study overall.

Overall incidence of gonococcal infection was almost a quarter higher in the nonoxynol-9 than in the placebo group (table 3), and overall incidence of chlamydial infection was slightly increased in the nonoxynol-9 group (p=0.37).

We assessed whether condom use and anal sex confounded our results. In Durban, 140 (75%) participants reported unprotected anal sex during follow-up, with 5060 (33%) of 15 255 anal acts in the nonoxynol-9 group and 3383 (19%) of 18 054 in the placebo group not protected by condoms. However, these findings did not seem to confound our results. Hazard ratio was 1.7 (95% CI 1.0-3.1) and 1.3 (0.8-2.3) in the groups with and without unprotected anal sex, respectively (p=0.42 for comparison of the two hazard ratios). For

	N gonorrhoeae					p	C trachomatis					p
	n	Woman-years	Number of events	Incidence per 100 woman-years	Hazard ratio (95% CI)*		n	Woman-years	Number of events	Incidence per 100 woman-years	Hazard ratio (95% CI)*	
Nonoxynol-9	356	263.2	106	40.3	1.2 (0.9-1.6)	0.19	353	280.9	72	25.6	1.2 (0.8-1.6)	0.37
Placebo	366	290.9	97	33.3	-	-	371	329.6	73	22.1	-	-

*Adjusted for centre.

Table 3: Incidence of sexually transmitted infections (time to first event)

	Nonoxynol-9			Placebo			Hazard ratio (95% CI)*	p
	Woman-years	Number of seroconversions	HIV-1 incidence per 100 woman-years	Woman-years	Number of seroconversions	HIV-1 incidence per 100 woman-years		
Gel use†								
Low (<1.5)	119.2	13	10.9	127.3	13	10.2	1.1 (0.54–2.4)	0.87
Medium (1.5–3.5)	174.5	13	7.4	169.1	11	6.5	1.3 (0.6–2.9)	0.56
High (>3.5)	107.8	33	30.6	137.5	20	14.5	1.8 (1.0–3.2)	0.03

*Adjusted for centre, anal sex, and condom use. †Reported mean applicator use per working day.

Table 4: HIV-1 incidence per treatment group and frequency of gel use

most vaginal sex acts, women reported condom use—ranging from 204 695 (74%) of 278 227 reported acts in Durban to 8580 (99%) of 8636 in Bangkok. Hazard ratio was 1.6 (0.5–5.7) for women who reported no unprotected vaginal sex, 1.3 (0.6–2.6) for women who reported a mean of 0.5 or fewer unprotected vaginal acts per working day, and 1.5 (0.9–2.5) for women who reported a mean of more than 0.5 acts ($p=0.95$ for comparison of the three hazard ratios). Our categories for unprotected vaginal sex were based on approximate tertiles.

Overall incidence of lesions, combining those with or without epithelial breach, did not differ significantly between the two treatment groups, 34.3% versus 29.4% in the nonoxynol-9 and placebo groups, respectively (relative risk 1.2; 95% CI 0.9–1.5; $p=0.80$).

To test our hypothesis of dose-dependent toxic effects of nonoxynol-9, we divided the mean gel use per working day into three categories based on tertiles. We assessed HIV-1 incidence per treatment group and per category of gel use. HIV-1 incidence increased most rapidly with increasing gel use in the nonoxynol-9 versus the placebo group (table 4). In the nonoxynol-9 group, HIV-1 incidence rose from 8.8 per 100 woman-years in women reporting mean use of 3.5 or fewer applicators per day to 30.6 in women reporting a higher mean daily use (hazard ratio 3.5; 95% CI 2.1–5.8, $p<0.0001$). In the placebo group, HIV-1 incidence in those categories was 8.1 and 14.5 per 100 woman-years, respectively (1.8; 1.0–3.3; $p=0.05$). The p value for the interaction between gel use and HIV-1 incidence was 0.50 (adjusted for centre, anal sex, and condom use).

We divided lesions into those with or without an epithelial breach and assessed whether incidence of lesions with a breach also increased with increasing gel use. We noted the same effect as above—ie, incidence of lesions rose with increasing gel use (table 5). The increase in incidence of lesions was seen in both groups, but it happened most rapidly in the nonoxynol-9 group. The p value for the interaction between gel use and lesions with breach was 0.11 (adjusted for centre, anal sex, and condom use).

Hazard ratio of HIV-1 infection in women who had at least one episode of a lesion with an epithelial breach (irrespective of treatment group) was twice that in those who never had such a lesion (2.2; 1.4–3.5, $p=0.0003$). Lesions without an epithelial disruption did not increase a woman's risk of HIV-1 infection (0.7; 0.4–1.4, $p=0.34$).

	Nonoxynol-9			Placebo			Hazard ratio (95% CI)*	p
	Woman-years	Number of seroconversions	HIV-1 incidence per 100 woman-years	Woman-years	Number of seroconversions	HIV-1 incidence per 100 woman-years		
Gel use†								
Low (<1.5)	117.4	8	6.8	119.4	11	9.2	0.9 (0.4–2.3)	0.85
Medium (1.5–3.5)	158.4	12	7.6	150.5	16	10.6	0.6 (0.3–1.2)	0.14
High (>3.5)	87.4	28	32.0	123.8	22	17.8	1.6 (0.9–2.8)	0.14

*Adjusted for centre, anal sex, and condom use. †Reported mean applicator use per working day.

Table 5: Incidence of lesions with an epithelial breach per treatment group and frequency of gel use

Discussion

Our results show that nonoxynol-9 increased risk of HIV-1 infection compared with placebo. Risk was especially high in women who used the study drug gel more than 3.5 times per day and who also had a high incidence of lesions with epithelial disruption. This finding suggests that nonoxynol-9 has an adverse effect on vaginal integrity when used frequently, thus increasing women's susceptibility to HIV-1 infection. At low frequency use, nonoxynol-9 had no effect, either positive or negative, on HIV-1 infection. This conclusion did not change after adjustment for sexual behaviour (frequency of vaginal sex not protected by condoms or unprotected anal sex).

Midway through our trial, the results of a randomised controlled trial with a nonoxynol-9 vaginal film became available, showing no effect on prevention of HIV-1 (rate ratio 1.0; 95% CI 0.7–1.5).¹⁶ This study was done in female sex workers who were asked to insert the film before every vaginal sexual act. An expert committee advised continuation of our trial since the gel we used is easier to apply and covers vaginal walls as well as the cervix. Furthermore, nonoxynol-9 in the gel is immediately available whereas the film must first dissolve, which could take too long in a sex-worker population. Moreover, the higher nonoxynol-9 dose in the film (72 mg) could have caused more lesions, possibly enhancing risk of HIV-1 infection.

An increasing frequency of lesions with an escalating dose of nonoxynol-9 accords with results of the study by Roddy and colleagues.¹³ We did not note a toxic effect of nonoxynol-9 in the safety studies we did before phase 3,^{14,15} probably because phase 2 studies are short in duration, and are underpowered to see the size of an effect that is noted in a phase 3 trial. Increasing frequency of lesions in the placebo group can be expected. Women who frequently use gels are highly sexually active and thus will have more lesions than women with low sexual activity.

The noted effect of nonoxynol-9 was consistent in three of the four phase 3 centres. The drug seemed to have a significant protective effect against HIV-1 infection in only one centre (Abidjan). The significant treatment by centre interaction is mainly attributable to extremes in centre-specific hazard ratios in Abidjan and Hat Yai, both with small numbers of events (five in total in each centre). We expected differences in treatment effect between centres, in view of the differences in study populations, condom use, and sexual behaviour. We

could find no explanation for the apparent protective effect of nonoxynol-9 in Abidjan, and judged it to be the result of random variation. The placebo we used in our study—Replens—could have had a protective effect against HIV-1, however our study was not designed to estimate the effectiveness of this placebo on HIV-1 risk.

We chose an open cohort design with continuous enrolment because of the high mobility of the study population, and this design allowed long-term follow-up of some women, kept the decrease of HIV-1 incidence with time to a minimum (a characteristic of a closed cohort design), and yielded a feasible sample size.

Some factors in study design could affect validity of results. The retention rate in the study was 68% after 48 weeks. Our trial was designed with the assumption of an annual retention rate of 60%. Apart from Durban, female sex workers are very mobile, and in west Africa they frequently travel between countries. In Cotonou for example, 80% of sex workers were foreign. We recorded that most participants who did not return for follow-up had moved either to another city or to another country. Furthermore, the adverse effect of nonoxynol-9 was seen in Durban (hazard ratio 1.6; 1.0–2.5), which was the largest centre in the study that had a retention rate of almost 90% after 48 weeks. Since HIV-1 infection is usually a silent endpoint and there is little morbidity at the time of seroconversion, we do not think that women lost to follow-up were more likely to be infected than those retained. However, since the rate of loss to follow-up was slightly higher in the nonoxynol-9 than the placebo group, if loss was associated with incident HIV-1 infection, the adverse effect of the study product would have been underestimated.

Some baseline differences between women who were lost to follow-up and women who stayed in the trial were recorded. Women who stayed in the trial were older, less educated, and had longer average duration of sex work at enrolment than were those who were lost to follow-up.

Although risk of HIV-1 infection in users of nonoxynol-9 was higher than in users of placebo, participants benefited from intensive safer-sex counselling and condom promotion activities. Although we do not have comparable data on HIV-1 incidence in women not included in the trial, data from Hlabissa (South Africa) on women attending antenatal clinics in 1998 (not female sex workers) showed HIV-1 incidence of 15 per 100 woman-years.¹⁷ In Abidjan, HIV-1 incidence was 16.5 per 100 woman-years in female sex workers who did not have any intervention, and 6.5 per 100 woman-years in those who were diagnosed with and treated for a sexually transmitted infection,¹⁸ compared with 3.7 per 100 woman-years in this centre in our study. Although direct comparisons are difficult, these data suggest that trial procedures helped to reduce risk of HIV-1 infection in study volunteers.

The absence of any effect of nonoxynol-9 gel on *N gonorrhoeae* and *C trachomatis* infections accords with results of two other randomised controlled trials of nonoxynol-9 of these gels.^{16,19}

Since data on sexual behaviour are observational, results of exploratory analyses have to be interpreted with some caution.

One concern about our study remains the validity of self-reported sexual behaviour data obtained by interview and diary cards. These limitations affect both study groups equally. The overall result based on the intent-to-treat analysis is independent of these data and shows the adverse effect of nonoxynol-9. Analyses of HIV-1 incidence rates on the basis of self-reported patterns of anal sex and frequency of condom use gave biologically plausible results.

In one centre (Cotonou) we noted that a batch of study gels became liquid during a period of the study (March 1, 1999, to July 15, 1999), and a new batch of gels was thus supplied. This drawback did not recur, nor was it seen in other centres. Analysis excluding the data from Cotonou during this period did not affect our conclusions. After breaking of the randomisation code, we noted that the nonoxynol-9 gels had become liquid, but the reasons remain unclear.

We did not give antiretroviral treatment to participants in our study. When we implemented the trial, this treatment was not available in the countries participating in this study. During the trial, the Trial Management Committee decided not to provide antiretrovirals because this could be a coercive factor on study participation, since treatment was not widely available in those countries.

In conclusion, nonoxynol-9 no longer has a part to play in HIV-1-prevention. Our data show that low frequency use of nonoxynol-9 causes neither harm nor benefit; but that frequent use increases a woman's risk of HIV-1 infection by causing lesions.

Although efforts to promote condoms should be increased, research on additional HIV-1-prevention methods, such as other female-controlled methods, microbicides, and vaccines, should be reinforced.

Contributors

L Van Damme was the overall study coordinator and was involved in protocol design, data management and interpretation, and in writing and editing the report. G Ramjee, V Chandeying, H Rees, P Sirivongrangsorn, L Mukenge-Tshibaka, S Abdool Karim, and M Alary were involved in protocol design, data collection, and revision of the report. M Alary was also involved in data analysis and interpretation. B Vuylsteke, V Ettiègne-Traoré, and C Uaheowitchai were involved in data collection and revision of the report. J Perriens contributed to protocol design and report revision. M Laga was involved in protocol design, data interpretation, and revision of the report. B Mâsse was the study statistician.

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Conflict of interest statement

None declared.

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References

- 1 Benes S, McCormack WM. Inhibition of growth of *Chlamydia trachomatis* by nonoxynol-9 in vitro. *Antimicrob Agents Chemother* 1985; 27: 724–26.

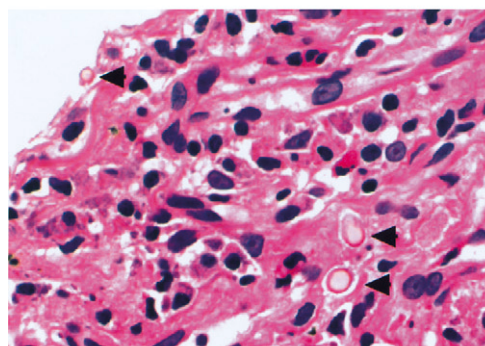
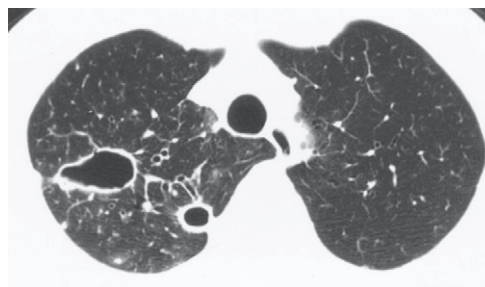
- 2 Bourinbaier AS, Fruhstorfer EC. The efficacy of nonoxynol-9 from an in vitro point of view. *AIDS* 1996; **10**: 558.
- 3 Hicks DR, Martin LS, Getchell JP, et al. Inactivation of HTLV-III/LAV-infected cultures of normal human lymphocytes by nonoxynol-9 in vitro. *Lancet* 1985; **2**: 1422–23.
- 4 Polsky B, Baron PA, Gold JWM, Smith JL, Jensen, RH, Armstrong D. In-vitro inactivation of HIV-1 by contraceptive sponge containing nonoxynol-9. *Lancet* 1988; **1**: 1456.
- 5 Kelly JP, Reynolds RB, Stagno S, Louv WC, Alexander WJ. In vitro activity of the spermicide nonoxynol-9 against *Chlamydia trachomatis*. *Antimicrob Agents Chemother* 1985; **27**: 760–62.
- 6 Miller CJ, Alexander NJ, Gettie A, Hendrickx AG, Marx PA. The effect of contraceptives containing nonoxynol-9 on the genital transmission of simian immunodeficiency virus in rhesus macaques. *Fertil Steril* 1992; **57**: 1126–28.
- 7 Louv WC, Austin H, Alexander WJ, Stagno S, Cheeks J. A clinical trial of nonoxynol-9 for preventing gonococcal and chlamydial infections. *J Infect Dis* 1988; **158**: 518–23.
- 8 Niruthisard S, Roddy RE, Chutivongse S. Use of nonoxynol-9 and reduction in rate of gonococcal and chlamydial cervical infections. *Lancet* 1992; **339**: 1371–75.
- 9 Roddy RE, Schulz KF, Cates W Jr. Microbicides, meta-analysis, and the N-9 question: where's the research? *Sex Transm Dis* 1998; **25**: 151–53.
- 10 Rosenberg MJ, Rojanapithayakorn W, Feldblum PJ, Higgins JE. Effect of the contraceptive sponge on chlamydial infection, gonorrhoea and candidiasis. *JAMA* 1997; **257**: 2308–12.
- 11 Zekeng L, Feldblum PJ, Oliver RM, Kaptue L. Barrier contraceptive use and HIV infection among high-risk women in Cameroon. *AIDS* 1993; **7**: 725–31.
- 12 Kreiss J, Ngugi E, Holmes KK, et al. Efficacy of nonoxynol-9 contraceptive sponge use in preventing heterosexual acquisition of HIV in Nairobi prostitutes. *JAMA* 1992; **268**: 477–82.
- 13 Roddy RE, Cordero M, Cordero C, Fortney JA. A dosing study of nonoxynol-9 and genital irritation. *Int J STD AIDS* 1993; **4**: 165–70.
- 14 Van Damme L, Niruthisard S, Atisook R, et al. Safety evaluation of nonoxynol-9 gel in women at low risk of HIV infection. *AIDS* 1998; **12**: 433–37.
- 15 Van Damme L, Chandeying V, Ramjee G, et al. Safety of multiple daily applications of COL-1492, a nonoxynol-9 vaginal gel, among female sex workers. *AIDS* 2000; **14**: 85–88.
- 16 Roddy RE, Zekeng L, Ryan KA, Tamoufé U, Weir SS, Wong EL. A controlled trial of nonoxynol-9 film to reduce male-to-female transmission of sexually transmitted diseases. *N Engl J Med* 1998; **339**: 504–10.
- 17 Williams B, Gouws E, Wilkinson D, Karim SA. Estimating HIV incidence rates from age prevalence data in epidemic situations. *Stat Med* 2001; **20**: 2003–16.
- 18 Ghys PD, Diallo MO, Ettiègne-Traoré V, et al. Effect of interventions to control sexually transmitted disease on the incidence of HIV infection in female sex workers. *AIDS* 2001; **15**: 1421–31.
- 19 Roddy RE, Zekeng L, Tyan KA, Tamoufé U, Tweedy KG. Effect of nonoxynol-9 gel on urogenital gonorrhoea and chlamydial infection. *JAMA* 2002; **287**: 1117–22.

Clinical picture

Chronic coccidioidomycosis

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A 40-year-old Japanese engineer with diabetes mellitus, who had been living in Phoenix, Arizona, for 14 years, presented with a 2-year history of dry cough and weight loss. High-resolution computed tomography showed thin-walled cavities and nodules with satellite lesions in the upper lobe of the right lung (figure, upper). We did a transbronchial lung biopsy and serological tests for fungus. On microscopic examination of the specimen of cavity wall we identified 5–10 µm fungi (haematoxylin and eosin; figure, lower). A quantitative complement fixation test showed a high titre for *Coccidioides immitis*, indicating extra-pulmonary dissemination. The main diagnostic difficulty in cavitary lung disease is neglecting to consider coccidioidomycosis.



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