Safety evaluation of nonoxynol-9 gel in women at low risk of HIV infection

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Objective: To determine the safety of a vaginal microbicide, COL-1492, containing 52.5 mg nonoxynol-9, applied once daily for 14 days among healthy volunteers.

Methods: A randomized, double-blind controlled trial with three arms, COL-1492 gel versus placebo gel versus no-treatment controls, was conducted. Outcomes of interest were reported genital symptoms, incidence of gynaecological signs, and incidence of genital lesions revealed by colposcopy. Participants were enrolled in four centres (Belgium, The Netherlands, and two in Thailand).

Results: A total of 534 women participated in the study: 179 used COL-1492, 178 used placebo, and 177 were no-treatment controls. Study visits were scheduled 1 week prior to enrolment (day −7), day 0 (enrolment), day 8 and day 14. The most frequently reported genital symptom was vaginal discharge in both the COL-1492 and placebo groups. This appeared to be related to leakage of the product out of the vagina. The incidence of lesions associated with epithelial disruption (ulcers and abrasions) was very low (< 2%) and there was no statistically significant difference between the three groups. Of the lesions observed by colposcopy that did not involve epithelial disruption, petechial haemorrhage was the most frequently detected, with an incidence of 20.1, 9.0 and 7.3% in the COL-1492, placebo and control groups, respectively. COL-1492 users had a higher incidence of erythema (8.4 versus 2% in the other groups).

Conclusion: COL-1492 showed minimal toxicity when applied once daily. A Phase III trial to assess the product's effectiveness in HIV prevention is currently ongoing.

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Introduction

The development of safe and effective microbicides capable of inactivating HIV and other sexually transmitted disease (STD) organisms in the vagina is a priority in order to widen the range of safer sex choices available to sexually active people and to empower women in the fight against AIDS [1–4]. Several clinical studies have shown that the non-ionic surfactant nonoxynol-9, which has been licensed for vaginal use as a spermicidal contraceptive for many years, can confer partial protection against gonococcal and chlamydial infections [5–7]. Nonoxynol-9 also has potent anti-HIV activity *in vitro* [8,9]. However, whereas two

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uncontrolled and fairly small studies suggested that it gave some protection against HIV infection *in vivo* [10,11], a randomized controlled trial on a vaginal sponge containing a very high dose of nonoxynol-9 (1000 mg) among female sex workers in Nairobi failed to show a protective effect [12]. This might be due to the high incidence of genital mucosal lesions, because these could in theory enhance transmission of HIV [13–16]. As a consequence of this trial, the safety of spermicides in settings in which HIV transmission is possible was questioned, and it was recommended that the local safety of candidate microbicides be established before large-scale efficacy studies on their ability to decrease HIV transmission were undertaken.

In this article we present the results of a safety study on COL-1492, a spermicidal gel that contains 52.5 mg nonoxynol-9 per dose, which is less than other spermicides containing this product as the active ingredient (range, 70–150 mg).

Methods

This study was a multicentre randomized controlled trial with three arms (COL-1492 gel versus placebo gel versus no-treatment control), double-blinded for product content for the COL-1492 and placebo arms. A separate randomization was undertaken at each centre.

Participants were recruited in four centres: the outpatient department at the STD/HIV Research and Intervention Unit, Institute of Tropical Medicine, Antwerp, Belgium; the Department of Obstetrics and Gynaecology, Chulalongkorn University Hospital, Bangkok, Thailand; the Department of Obstetrics and Gynaecology, Siriraj University Hospital, Mahidol University, Bangkok, Thailand; and the Department of Obstetrics and Gynaecology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands. Healthy female volunteers who met the following inclusion criteria were eligible for study participation: low risk of HIV infection (determined by interview); age between 18 and 45 years; normal pelvic examination; no clinical or colposcopic abnormalities; no evidence of STD. Sexually active participants were enrolled only if they were using a reliable method of contraception (hormonal, male condoms, intrauterine device or sterilization). Exclusion criteria were current use of a female-controlled barrier contraceptive method or spermicide, pregnancy or STD. At enrolment, participants were required to be within 1 week of completion of their most recent menses to enable evaluation after 2 weeks without interference by menses. Written informed consent was obtained from all participants. Participants were permitted to have sexual intercourse during the trial.

COL-1492 gel (marketed in the United States as the spermicidal gel Advantage 24, Columbia Laboratories, New York, New York, USA) contains 3.5% nonoxynol-9; other constituents include polycarbophil, a polymer with bioadhesive properties. The placebo gel was a similar product but without nonoxynol-9. Both were packed in single-dose, disposable plastic applicators designed to deliver 1.5 g gel. COL-1492 and the placebo were provided by Columbia Laboratories (Paris, France). Women randomized to one of the treatment arms were instructed to apply the relevant study product intravaginally once daily at about the same time every day for 14 days. All participants were requested not to apply vaginal douches, or to insert tampons or any other object into the vagina. Empty and unused applicators were returned to the trial centres to assess compliance.

Clinical evaluations were scheduled at days 0, 7 and 14. In addition, participants were requested to attend for evaluation if they experienced any health problem between scheduled visits. At each visit a history was obtained and colposcopy was performed. Whenever a genital lesion was diagnosed, an additional visit was arranged so that its evolution could be recorded.

The main outcomes of interest were the genital symptoms reported, the incidence of gynaecological signs, and the incidence of genital lesions revealed by colposcopy. Lesions detected at colposcopy were described as recommended by the World Health Organization (WHO) [17].

Ulcerations or abrasions led to an immediate discontinuation of study drug use. When ecchymosis, petechial haemorrhage, subepithelial swelling or erythema occurred, study drug use was continued but the participant was re-evaluated after 3 days. If the lesions worsened, study drug use was discontinued.

An intention-to-treat analysis was undertaken on the data from women who used the study product at least once. Intergroup comparability was assessed using the χ^2 test and Fisher's exact test when appropriate. The log-rank test was used to determine the significance of any differences between the incidence of lesions in each group. All P values reported were two-tailed (except for mean age, which was based on analysis of variance), and a P value of 0.05 was regarded as statistically significant.

Results

Study population

Between 22 November 1993 and 16 January 1995, 562 volunteers were recruited: 170 in Antwerp, 101 in

Table 1. Baseline characteristics of the study population.

	Control	Placebo	COL-1492
No. women			
Randomized	187	188	187
Without follow-up data	3	2	6
With abnormal colposcopy on enrolment	7	8	2
Analysed	177	178	179
Mean (SD) age (years)	31 (6.7)	31 (7.6)	30 (7.4)
Symptoms			
Vaginal discharge*	18	19	13
Other	6	9	6
Physical findings			
Vaginal discharge*	16	18	13
Cervical discharge*	76	63	69

*Not considered to be abnormal in quantity or aspect.

Amsterdam, 129 at the Chulalongkorn University, Bangkok, and 131 at the Siriraj Hospital, Bangkok. Twenty-eight participants were excluded from the analysis: there were no follow-up data for 11 participants, and 17 participants had abnormal colposcopic findings at enrolment. The analysis was carried out on data from 534 participants, divided evenly over the three study groups. These groups were similar for all characteristics analysed at baseline (Table 1).

Of the 534 women included in the analysis, 499 (93.4%) completed the study. Participants in the COL-1492 group were less likely to complete the study (88.2%) than women in the placebo group (93.2%) or in the no-treatment control group (98.8%), mainly because they discontinued treatment more often because of lesions than those in the placebo or no-treatment groups ($P = 0.04$ and 0.007, respectively).

Incidence of genital symptoms and gynaecological signs

The incidence of genital symptoms was significantly greater in the COL-1492 group than in the placebo group (except for genital itching), and in the placebo group than in the no-treatment control group. The most frequently reported symptom was vaginal discharge (Table 2). Vaginal discharge was the only gynaecological sign to be more frequent in the COL-1492 group than in the placebo and no-treatment control groups (Table 2).

Incidence of abnormalities revealed by colposcopy

Women in the COL-1492 group were significantly more likely ($P < 0.001$) to develop a lesion than those in either the placebo or no-treatment control group. Petechial haemorrhage was the most frequent abnormality reported (Table 3). Its incidence in the COL-1492 group was 20.1%, significantly greater than in the placebo group (9%) or the control group (7.3%). Erythema also occurred significantly more frequently in the COL-1492 group (8.4% incidence) than in the placebo group (1.7%) and the no-treatment group (2.3%). The incidence of oedema was 7.3% in the

Table 2. Incidence of gynaecological symptoms and signs during the study period.

	No. reports/total (%)			*P*		
	Control	Placebo	COL-1492	COL-1492 versus placebo	COL-1492 versus control	Placebo versus control
Symptoms*						
Vaginal discharge	9/159 (5.7)	30/159 (19.0)	53/166 (32.0)	0.01	< 0.001	< 0.001
Genital itching	3/175 (1.7)	15/173 (8.7)	25/174 (14.4)	0.13	< 0.001	0.003
Other symptoms	5/172 (2.9)	14/166 (8.4)	31/174 (17.8)	0.02	< 0.001	0.03
Signs*						
Vaginal discharge	28/161 (17.4)	31/160 (19.4)	50/166 (30.1)	0.03	< 0.01	0.67
Cervical discharge	17/101 (16.8)	18/115 (15.7)	24/110 (21.8)	0.31	0.39	0.86
Other signs	19/176 (10.8)	13/178 (7.3)	14/179 (7.8)	1.00	0.37	0.27

*Amongst those women who did not have the symptom/sign at enrolment.

Table 3. Incidence of colposcopic lesions during the study.

	No. reports (%)			*P*		
	Control (n = 177)	Placebo (n = 178)	COL-1492 (n = 179)	COL-1492 versus placebo	COL-1492 versus control	Placebo versus control
Ulcer	0 (0)	0 (0)	3 (1.7)	0.25	0.25	–
Abrasion	2 (1.1)	5 (2.8)	3 (1.7)	0.50	1.00	0.45
Ecchymosis	0 (0)	1 (0.6)	2 (1.1)	1.00	0.50	1.00
Petechial haemorrhage	13 (7.3)	16 (9.0)	36 (20.1)	0.004	< 0.001	0.70
Subepithelial haemorrhage and swelling	0 (0)	0 (0)	3 (1.7)	0.25	0.25	–
Erythema	4 (2.3)	3 (1.7)	15 (8.4)	0.006	0.02	0.72
Oedema	0 (0)	6 (3.4)	13 (7.3)	0.16	< 0.001	0.03
Other	18 (10.2)	26 (14.6)	54 (30.2)	< 0.001	< 0.001	0.26

COL-1492 group compared with 3.4% in the placebo group. This difference did not achieve statistical significance, although the difference between both of these groups and the no-treatment group (in which there were no cases of oedema) was significant.

The incidence of ulceration and abrasion, lesions that constitute a breach in the genital mucosa, was low in all groups (0–3%), and the differences between the three groups were not statistically significant. When lesions occurred, no significant differences were apparent between the three groups of women in terms of healing on discontinuation of the study products. The incidence of STD or other genital infections was low, and similar in the three study groups (data not shown).

Discussion

The results reported indicate that the use of COL-1492 gel once daily for 14 consecutive days is not associated with a significant level of lesions with an epithelium disruption. As observed in previous studies [18,19], most of the lesions were in the vagina and on the cervix. The great majority of lesions healed fairly quickly on discontinuation of the product, a phenomenon that has been reported previously [19].

Ulcerations may enhance HIV transmission [13–16,20]. Lesions with epithelial disruption were observed only very infrequently (incidence of ulcers and abrasions, both <2%) and for these types of lesion there were no statistically significant differences between the COL-1492, placebo and no-treatment control groups. The overall frequency of epithelia-disrupting lesions in the COL-1492 group (3.4%) was considerably lower than the frequency reported from a study of a suppository containing 150 mg nonoxynol-9 once daily (34%) [18]. This result is consistent with the low risk of epithelial disruption associated with nonoxynol-9 use observed by Martin *et al.* [21] among female sex workers in Kenya. Although Roddy *et al.* [22] found a higher incidence of genital lesions among vaginal contraceptive film users compared with placebo, this difference was not significant.

The clinical significance of the excess incidence of petechial haemorrhage in the COL-1492 group, and of other types of lesion not associated with epithelial disruption, is unclear. In theory, it is possible that any type of genital lesion might increase the woman's risk of becoming infected with HIV, especially if it serves as a focus for the recruitment of HIV-infectable inflammatory cells. Whether this is in reality a significant factor and one that could outweigh the potential benefits of COL-1492 in preventing HIV transmission will only be ascertained by means of large-scale intervention trials in populations at high risk of HIV infection.

The carrier material in COL-1492 gel appears to be fairly innocuous. In terms of lesions, the only significant difference between the placebo group and the control group was a higher incidence of oedema in the former. There was also a significantly higher incidence of reported vaginal discharge and genital itching in the placebo group. This may be at least partially related to leakage of the product out of the vagina.

The data obtained from the no-treatment group provided us with information on the changes or lesions occurring in sexual active women who were not using any intravaginal product. They may be used as a comparison group in future vaginal microbicide safety trials.

We used colposcopy to assess genital lesions, and although we followed the WHO manual [17], we were aware of the limitations of a visual and therefore, by definition, a subjective diagnostic tool.

Sexual intercourse was allowed during the study period to reflect the real life situation, as an HIV prevention method of this type will only be used by sexually active women. However, we acknowledge that we cannot be sure what percentage of lesions diagnosed in the treatment arm versus the no-treatment arm were due to product toxicity and not to sexual intercourse.

In this study, women were asked to apply the product once daily and the findings suggested that at this frequency the use of COL-1492 can be considered safe. However, trials of the product's effectiveness in the field will inevitably need to be conducted among women at high risk of HIV infection (e.g., female sex workers). These women will probably use the product several times daily. Having established the safety of a single dose application of COL-1492, it was important to evaluate the effects of multiple doses. This was the objective of a recently completed study involving the application of COL-1492 four times daily (unpublished data). The findings encouraged the further testing of COL-1492. Consequently, UNAIDS has decided to sponsor a Phase II/III trial to assess the product's effectiveness in preventing male-to-female HIV transmission. This study is currently ongoing in several sites in Africa and one in Thailand.

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References

1. Stein ZA: **HIV prevention: the need for methods women can use.** *Am J Public Health* 1990, **80**:460–462.
2. Global Programme on AIDS, World Health Organization: *Report on a Meeting on the Development of Vaginal Microbicides for the Prevention of Heterosexual Transmission of HIV [Document WHO/GPA/RID/CRD/94.1;11–13 November 1993].* Geneva: GPA/WHO; 1993.
3. Stone AB, Hitchcock PJ: **Vaginal microbicides for preventing the sexual transmission of HIV.** *AIDS* 1994, **8** (suppl 1):S285–S293.
4. Elias CJ, Heise LL: **Challenges for the development of female-controlled vaginal microbicides.** *AIDS* 1994, **8**:1–9.
5. Weir SS, Feldblum PJ, Zekeng L, Roddy RE: **The use of nonoxynol-9 for protection against cervical gonorrhea.** *Am J Public Health* 1994, **84**:910–914.
6. Niruthisard S, Roddy RE, Chutivongse S: **Use of nonoxynol-9 and reduction in rate of gonococcal and chlamydial cervical infections.** *Lancet* 1992, **339**:1371–1375.
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**:518–523.
8. Malkovsky M, Newell A, Dalgleish AG: **Inactivation of HIV by nonoxynol-9 [letter].** *Lancet* 1988; **i**:645.
9. Hicks DR, Martin LA, Getchell JP, *et al.*: **Inactivation of HTLV-II/LAV-infected cultures of normal human lymphocytes by nonoxynol-9 *in vitro*.** *Lancet* 1985, **ii**:1422–1423.
10. Zekeng L, Feldblum PJ, Oliver RM, Kapute L: **Barrier contraceptive use and HIV infection among high-risk women in Cameroon.** *AIDS* 1993, **7**:725–731.
11. Feldblum P, Hira S, Goodwin S, Kamanga J, Mukelabaie G: **Efficacy of spermicide use and condom use by HIV-discordant couples in Zambia.** *VIII International Conference on AIDS/III STD World Congress*. Amsterdam, July 1992 [abstract WeC1085].
12. Kreiss J, Ngugi E, Holmes K, *et al.*: **Efficacy of nonoxynol-9 contraceptive sponge use in preventing heterosexual acquisition of HIV in Nairobi prostitutes.** *JAMA* 1992, **268**:477–482.
13. Cameron DW, Lourdes JD, Gregory MM, *et al.*: **Female to male transmission of human immunodeficiency virus type 1: risk factors for seroconversion in men.** *Lancet* 1989, **ii**:403–407.
14. Plummer FA, Simonsen JN, Cameron DW, *et al.*: **Co-factors in female-to-male sexual transmission of HIV.** *J Infect Dis* 1991, **163**:233–239.
15. Plourde PJ, Pepin J, Agoki E, *et al.*: **Human immunodeficiency virus type 1 seroconversion in women with genital ulcers.** *J Infect Dis* 1994, **170**:313–317.
16. Ghys PD, Diallo MO, Ettiegne-Traore V, *et al.*: **Genital ulcers associated with human immunodeficiency virus-related immunosuppression in female sex workers in Abidjan, Ivory Coast.** *J Infect Dis* 1995, **172**:1371–1374.
17. WHO/GPA/RID/CRD: *Manual for the Standardization of Colposcopy for the Evaluation of Vaginally Administered Products.* Geneva: World Health Organization; 1995.
18. Roddy RE, Cordero M, Cordero C, Fortney JA: **A dosing study of nonoxynol-9 and genital irritation.** *Int J STD AIDS* 1993, **4**:165–170.
19. Niruthisard S, Roddy RE, Chutivongse S: **The effects of frequent nonoxynol-9 use on the vaginal and cervical mucosa.** *Sex Transm Dis* 1991, **18**:176–179.
20. Weir SS, Roddy RE, Zekeng L, Feldblum PJ: **Nonoxynol-9 use, genital ulcers, and HIV infection in a cohort of sex workers.** *Genitourin Med* 1995, **71**:78–81.
21. Martin HL, Stevens CE, Richardson BA, *et al.*: **Safety of a nonoxynol-9 vaginal gel in Kenyan prostitutes.** *Sex Transm Dis* 1997, **24**:279–283.
22. Roddy RE, Zekeng L, Ryan KA, Tamoufe A, Weir SS: **A randomized controlled trial of the effect of nonoxynol-9 film use on male-to-female transmission of HIV-1.** *National Conference on Women and HIV.* Pasadena, May 1997 [abstract 215.3].