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https://escholarship.org/uc/item/993928r7

Journal

Contraception, 74(2)

ISSN

0010-7824

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Publication Date

2006-08-01

DOI

10.1016/j.contraception.2006.02.008

Peer reviewed

Original research article

Fourteen-day safety and acceptability study of 6% cellulose sulfate gel: a randomized double-blind Phase I safety study

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Abstract

Background: Topical microbicides against the human immunodeficiency virus (HIV) 1 that are nonirritating to the female genital epithelium are urgently needed to slow the heterosexual spread of HIV infection. Products that are also effective contraceptives provide additional benefits. Cellulose sulfate (CS) is a noncytotoxic antifertility agent that exhibits in vitro antimicrobial activity against sexually transmitted pathogens, including HIV.

Methods: We performed a multicenter, Phase I, placebo-controlled, randomized study to evaluate the genital toxicity of CS. Two cohorts of healthy women used 3.5 ml of 6% CS gel or 3.5 ml of K-Y Jelly, vaginally, bid, for 14 days. The first cohort was sexually abstinent, and the second cohort was sexually active.

Results: CS was associated with only a slightly higher odds ratio (OR) of symptoms of minor urogenital irritation compared to the inactive lubricant K-Y Jelly (OR=2.02, 95% confidence interval=0.90–4.53). In addition, there were minor shifts in some genital flora, but there was no evidence of greater inflammation as evidenced by few colposcopic findings, decreased influx of polymorphonuclear cells and minimal changes in proinflammatory cytokines. Moreover, both products appeared acceptable to most women. Product leakage was identified as more of a problem in sexually abstinent women, but less so in women using the product for sexual intercourse, as would be the case in actual practice.

Conclusion: CS was safe for twice-daily use for 14 days. CS is appropriate for future studies in effectiveness trials.
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Keywords: Cellulose sulfate; Contraception; Microbicide; Sexually transmitted infection; Clinical trial

1. Introduction

About half of all human immunodeficiency virus (HIV) infections worldwide are found in women, yet the only approved female-initiated barrier method with claims of reduction of sexually transmitted pathogen transmission, including HIV, remains the female condom. Microbicides are vaginally applied chemical barriers that offer women who at risk for HIV and other sexually transmitted infections (STIs) an HIV-reducing method not requiring the cooperation of their partners. The spermicide nonoxynol-9 (N-9), a surfactant that destroys cellular and microbial membranes, is an effective contraceptive [1]. However, N-9 is not effective for the prevention of HIV-1 and other STIs, and, with frequent use, may increase a woman's risk due to genital epithelial disruption [2]. Alternative methods — such as vaginal microbicides, whose antimicrobial effects are not mediated by nonspecific cytotoxic mechanisms and are, therefore, nonirritating to the genital epithelium and

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compatible with the mucosal immune barrier — are urgently needed to slow the spread of heterosexual HIV infection.

Cellulose sulfate (CS) is such a noncytotoxic microbicide that functions as an entry inhibitor. It exhibits antimicrobial activity against sexually transmitted pathogens, inhibits sperm function in vitro [3] and acts as a contraceptive when applied vaginally in rabbits [4]. CS blocks cell surface receptors, inhibits the HIV binding and penetration of epithelial layers and dendritic cells, blocks the gp120–CD4 coreceptor interaction and acts against coreceptors CCR5 and CXCR4 in primary isolates and laboratory-adapted strains [5].

A 6-day study conducted on women in the United States suggested that 6% CS was as safe and acceptable as the control, K-Y Jelly [6]. Similarly, CS was found to be safe when applied to the penis for seven consecutive days [7]. A magnetic resonance imaging study of the distribution of CS gel showed that a volume of 3.5 ml, compared to a volume of 2.5 ml, improved spread throughout the vagina without significant leakage [8].

The objective of this study was to assess whether CS is safe enough in preserving genital epithelial integrity and function, in comparison to a marketed sexual lubricant, to warrant further development.

2. Materials and methods

This was a randomized double-blind Phase I study performed at three centers: the CONRAD Clinical Research Center at the Eastern Virginia Medical School (Norfolk, VA, USA), the Magee-Women's Hospital (Pittsburgh, PA, USA) and Profamilia (Santo Domingo, Dominican Republic). Procedures were reviewed with the study personnel for all three centers during investigators' meeting prior to the start of the study. Sample size was based on convenience, as appropriate for a Phase I vaginal product safety study. Across all sites, 30 women were enrolled in each of two treatment groups. The study was first conducted within a cohort of 30 sexually abstinent women before being conducted in a cohort of 30 sexually active couples. An independent data monitoring committee reviewed safety data when a site completed the sexually abstinent cohort and was ready to progress to the sexually active cohort. All study sites progressed to the sexually active portion of the study. Appropriate human subjects approval was obtained at each center.

The formulations tested were 3.5 ml of 6% CS gel (150 mg of CS)[1] and 3.5 ml of K-Y Jelly.[2] All gels were packaged in identical, single-use applicators manufactured by HTI Plastics (Lincoln, NE, USA).

Healthy female volunteers who were 18–50 years old, were in good health, had regular menstrual cycles, had a

normal Pap smear, were not at risk for pregnancy and provided signed informed consent were enrolled. Women in the sexually active cohort were required to have had a mutually monogamous relationship for at least 3 months and to have been protected from pregnancy by either male or female sterilization or a nonintravaginal hormonal contraceptive. Nonpregnant female volunteers without vaginal candidiasis, bacterial vaginosis (BV) and/or urinary tract infection, and with normal ($\pm20\%$) complete blood count (CBC), chemistry panel and activated partial thromboplastin time were enrolled. Female volunteers were ineligible if they have had a hysterectomy, were breastfeeding or were within 2 months from their last pregnancy outcome, or were allergic to the study product N-9 or latex (for the sexually active cohort). Female volunteers who tested positive for *Trichomonas vaginalis*, *Neisseria gonorrhoeae* or *Chlamydia trachomatis*; or who were recently diagnosed with, or treated for, any STI or pelvic inflammatory disease; or who had genital symptoms were ineligible. A history of drug or alcohol abuse, chronic or current use of antibiotics, or prior exposure to CS precluded participation.

Male volunteers were eligible for enrollment if they were at least 18 years old, provided signed informed consent and had been in a mutually monogamous relationship for at least 3 months with an eligible female partner. Male participants were ineligible if they were allergic to the study product N-9 or latex; if they had been recently diagnosed with, or treated for, any STI; or if they had recent symptoms consistent with an STI.

Random allocation sequences were created by a statistician not otherwise involved in the study, using a program based on the SAS[3] (SAS Institute, Inc., Cary, NC, USA) RANUNI function. Randomization employed the random permuted blocks method and was stratified by center and by cohort. Allocation sequences were provided to the product supplier, which packaged the products into identical white wrappers identified by participant number. A data check confirmed that participants were randomized in chronological order.

Each female volunteer was seen in four visits. On the screening visit, volunteers were instructed to abstain from all vaginal activities beginning 72 h prior to the enrollment visit, which was scheduled to fall within the follicular phase (Cycle Days 5–10) of the menstrual cycle. All colposcopies were carried out according to the World Health Organization/CONRAD Manual for the Standardization of Colposcopy for the Evaluation of Vaginally Administered Products Update 2000 [9].

On the enrollment visit, eligible volunteers were randomized to receive one of the two study products and were given 27 applicators for twice-daily use, at approximately 12-h intervals, for 14 days, excluding the morning of the third visit. The first application took place in the clinic,

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[1] The CS gel contained the following inactive ingredients: glycerin, methylparaben, propylparaben and water.

[2] The K-Y Jelly (Personal Products Co., Skillman, NJ, USA) contained chlorhexidine gluconate, methylparaben gluconodelta lactone, glycerin, hydroxyethyl cellulose, purified water and sodium hydroxide.

[3] SAS is a registered trademark of SAS Institute, Inc., in the United States and in other countries.

followed 15 min later by colposcopy. The sexually active participants were instructed to have vaginal intercourse, using the male condoms (nonspermicidal) provided, at least twice weekly and to time the intercourse in conjunction with the last gel insertion at least 6 h prior to the third and fourth visits. The third and fourth visits were scheduled to take place within 6–18 h after the 14th and final gel application, respectively.

A vaginal smear was obtained from the vaginal wall for Gram stain, air-dried on a glass slide and transported to the Magee-Women's Research Institute (MWRI) according to standard procedures for Nugent scoring [10]. Smears were also examined for the number of polymorphonuclear leukocytes (PMN) to evaluate for increases in inflammatory cells with product use. The score used a point scale from 0 to 4+ in the slide examination of five fields under oil immersion ($\times$1000) [(0)=*no PMN/field*; (1+)=*<1 PMN/field*; (2+)=*1–4 PMN/field*; (3+)=*5–30 PMN/field*; (4+)=*>30 PMN/field*].

A second swab was obtained for semiquantitative vaginal cultures for 11 organisms and was placed in anaerobic media (Port-a-Cul; Becton Dickinson Corp., Cockeysville, MD, USA) and transported overnight to the MWRI, where it was evaluated according to a previously published procedure [11]. Semiquantitative growth has been correlated with quantitative log growth according to the scale: (1+)= 10^2–10^4; (2+)=10^5; (3+)=10^6; (4+)=10^7–10^8 [12].

The vaginal walls and cervix were lavaged with 10 ml of normal saline. The collected fluid was centrifuged for 10 min at 1000$\times$g, after which the supernatant was frozen at -70 °C. Cytokine data were not collected from participants in the Dominican Republic. Cytokine analysis was performed at the Brigham and Women's Hospital (Boston, MA, USA). Standardized procedures were developed for the measurement of cytokines TNF-α, IL-1α, IL-1β, IL-1 receptor antagonist (IL-1RA) and IL-8 in saline cervical vaginal lavage (CVL) using commercial photometric or luminescence ELISA (R&D Systems and Endogen-Pierce, Rockford, IL, USA), a multilabel microplate counter Victor 2 (Perkin Elmer Life Sciences, Boston, MA, USA) and WorkOut Version 1.5 Wallace Software (DAZDAQ Ltd., Brighton, East Sussex, England, UK). Cytokine standards were recovered from CS gel and K-Y Jelly solutions to determine product interference with cytokine detection systems. The intra-assay and interassay coefficients of variation of cytokine recovery from the CVL matrix were determined by standard spiking in pooled CVLs. The criteria for elevated cytokine values were defined as moderate (mean at baseline+1 SD) and high (mean at baseline+2 SD).

Study objectives were evaluated in the treated population (women providing at least some follow-up data). The primary safety outcome was the proportion of women with any evidence (signs and symptoms) of urogenital irritation during 14 days of twice-daily use. Signs (as seen on pelvic examination and colposcopy) and symptoms (as reported by subjects; i.e., genital pruritus, genital and pelvic pain,

abnormal bleeding and other/vaginal lacerations) were evaluated as separate sub-endpoints. Determination of which symptoms were considered evidence of urogenital irritation was carried out by a clinician not performing study procedures and who reviewed blinded event descriptions. All noniatrogenic, new or worsening colposcopy findings were counted as signs of urogenital irritation.

The primary outcome was assessed separately for three intervals: immediately (15 min) after initial product application, after 7 days of product use and after an additional 7 days of product use, although the analysis evaluated the outcome across all intervals. The secondary outcomes were differences in vaginal health, as assessed by wet mounts, Gram stains, vaginal cultures and cytokine analyses. For safety endpoints measured at multiple time points (other than cytokine measurements), product groups were statistically compared using generalized estimating equation (GEE) models implemented by the GENMOD procedure in SAS, which controls for center and cohort, and treats time of measurement as a repeated measure. The analysis used the link function of logit for binary endpoints, cumulative logit for ordinal endpoints and gamma for one continuous endpoint (pH). Because of the small sample size, GEE comparisons with p<.15 were further evaluated using exact measures (data not shown). Cytokine data were compared between groups using exact Wilcoxon–Mann–Whitney tests stratifying on center and cohort, although a post hoc analysis used GEE. Adverse events (AEs) were coded using MedDRA version 5.1 (International Federation of Pharmaceutical Manufacturers Associates, Geneva, Switzerland). For symptoms reported by more than four women, proportions of women experiencing these AEs were compared between groups using an exact Mantel–Haenszel test pooling center and cohort.

The acceptability of the product was assessed using a questionnaire. The key acceptability item was prespecified as whether or not the participant said she would buy the product for either contraceptive use or for STI prevention. Proportions were compared between groups using exact logistic regression controlling for center and cohort.

3. Results

Recruitment occurred from October 2002 to September 2003; follow-up ended on October 2003. Sixty women, 30 in each treatment group, were enrolled. In the sexually abstinent cohort, 16 volunteers were randomized to CS gel and 14 were randomized to K-Y Jelly; the imbalance occurred because one set of supplies was tampered with by customs officials and removed. In the sexually active cohort, 14 volunteers were randomized to CS gel and 16 were randomized to K-Y Jelly; the imbalance occurred because the sequence had been generated balanced across 14 possible participants per cohort per site, but not across the 10 participants actually enrolled. Since all 60 women provided data and since there were no allocation errors, the treated population is identical to the intent-to-treat population.

Table 1
Participant characteristics (pooling cohorts)

Characteristics	CS (n=30)	K-Y (n=30)
Age [mean+SD (range)]	30+7.3 (18–49)	30+6.9 (19–44)
Education (school years completed) (mean+SD)	13+3.6	13+3.6
Ethnicity [n (%)]		
Hispanic/Latina	9 (30.0)	11 (36.7)
Not Hispanic/Latina	21 (70.0)	19 (63.3)
Race [n (%)]		
Black	2 (6.7)	1 (3.3)
White	19 (63.3)	18 (60.0)
More than one race/other	9 (30.0)	11 (36.7)
Partner status [n (%)]		
Living with partner	20 (66.7)	23 (76.7)
Not living with partner	5 (16.7)	5 (16.7)
No partner	5 (16.7)	2 (6.7)
Prior use of spermicide [n (%)]	24 (80.0)	27 (90.0)

Nineteen of 30 (63%) participants in the CS group compared to 15 of 30 (50%) in the K-Y group had at least one protocol violation, which was a minor deviation that did not affect the results or overall safety of the study. All participants completed the study using their assigned product at least 25 times out of a total of 27 expected applications and were seen at all scheduled visits, except for two participants who used only 14 applicators because they discontinued after 1 week: one (CS sexually abstinent group) who requested discontinuation due to moderate discomfort and one (K-Y sexually abstinent group) who discontinued after 1 week of product use because customs officials tampered with supplies. All participants in the sexually active cohort reported four to six coital acts during follow-up, and all but one of the sexually active participants (K-Y group) reported using condoms for each act of intercourse. Participant characteristics are summarized in Table 1.

3.1. Signs and symptoms of urogenital irritation

Overall, about equal percentages of women, 10 of 30 (33%) in the CS group and 8 of 30 (27%) in the K-Y group, reported 20 and 13 symptoms of urogenital irritation, respectively (Table 2). The most common symptom of urogenital irritation was pain, which accounted for more

than half of the complaints. Three participants assigned to the CS group experienced very slight or slight intermenstrual bleeding: (a) spotting related to oral contraceptives; (b) postexamination spotting unrelated to product; and (c) blood-tinged discharge possibly related to product.

One participant (CS group) complained of moderate pelvic pain from study examination and moderate vaginal discomfort during the insertion of study applicators. All other urogenital complaints were considered of very slight or slight severity. Symptoms of urogenital irritation were more commonly reported by participants in the sexually active group compared to the sexually abstinent cohort (Table 2). There were no serious, unexpected or product-use-related adverse experiences.

Nineteen (63%) participants in the CS group had 37 colposcopic findings, and 14 participants (47%) in the K-Y group had 28 colposcopic findings (Table 3). No colposcopic finding with deep epithelial disruption was observed at any point during this study. Each product group had five participants with at least one finding of superficial epithelial disruption. Of the 65 noniatrogenic findings observed during follow-up, 57 were considered by site investigators to be at least possibly product-related. The most common colposcopic finding was erythema, which predominated in sexually abstinent women regardless of product group and accounted for the greater number of findings in the abstinent women compared to that in the active women.

Across all product use periods, participants in the CS group tended to have slightly higher odds of experiencing any evidence of urogenital irritation compared to those in the K-Y group [odds ratio (OR)=1.36, 95% confidence interval (95% CI)=0.70–2.61]. This increase was predominantly based on an increase in the symptoms (OR=2.02, 95% CI=0.90–4.53) rather than in the signs (OR=1.18, 95% CI=0.62–2.22) of urogenital irritation (Table 4).

3.2. Microflora

One clinical diagnosis of BV was made (CS group) during the study and, as per the analysis plan, was not counted toward the endpoint of urogenital irritation. Only one participant (K-Y group) developed an abnormal Nugent

Table 2
Urogenital AEs

AE	Sexually abstinent				Sexually active			
	CS (n=16)		K-Y (n=14)		CS (n=16)		K-Y (n=14)	
	Events (n)	Women [n (%)]	Events (n)	Women [n (%)]	Events (n)	Women [n (%)]	Events (n)	Women [n (%)]
Pain[a]	4	3 (19)	1	1 (7)	8	5 (36)	6	4 (25)
Genital pruritus	2	2 (13)	0	0 (0)	2	1 (7)	5	4 (25)
Vaginal bleeding	2	1 (6)	0	0 (0)	2	2 (14)	0	0 (0)
Other (vulvar laceration)[b]	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (6)
Total number of events/women with at least one event[c]	8	4 (25)	1	1 (7)	12	6 (43)	12	7 (44)

[a] Includes genital burning, irritation, urinary symptoms, discomfort, dyspareunia, pelvic pain and increased sensitivity.
[b] Counted as a colposcopic finding and as a urogenital event in the analysis because it was reported by the participant and was seen on examination.
[c] The total number of women may not equal the column sum because a given woman may have more than one type of urogenital event.

Table 3
Colposcopic findings

	Sexually abstinent				Sexually active			
	CS (n=16)		K-Y (n=14)		CS (n=16)		K-Y (n=14)	
	Findings (n)	Women [n (%)]	Findings (n)	Women [n (%)]	Findings (n)	Women [n (%)]	Findings (n)	Women [n (%)]
Erythema	15	9 (56)	12	4 (29)	1	1 (7)	0	0 (0)
Petechiae[a]	7	3 (19)	4	1 (7)	3	3 (21)	5	3 (19)
Ecchymoses[a]	0	0 (0)	1	1 (7)	1	1 (7)	1	1 (6)
White lesion	0	0 (0)	0	0 (0)	1	1 (7)	0	0 (0)
Nodule	0	0 (8)	0	0 (0)	1	1 (7)	0	0 (0)
Peeling[b]	0	0 (0)	1	1 (7)	2	1 (7)	1	1 (6)
Abrasion[b]	1	1 (6)	0	0 (0)	0	0 (0)	0	0 (0)
Ulcer[b]	1	1 (6)	1	1 (7)	0	0 (0)	0	0 (0)
Laceration[b]	3	1 (6)	0	0 (0)	1	1 (7)	2	2 (13)
Total number of findings/women with at least one finding[c]	27	10 (63)	19	8 (57)	10	9 (64)	9	6 (38)

[a] Findings with disrupted blood vessels.

[b] Findings with disrupted epithelium, all of which were considered superficial.

[c] The total number of women may not equal the column sum because a given woman may have more than one type of finding.

score (8) during the study. Since there were very few positive outcomes, comparison statistics could not be computed on yeast, BV or *T. vaginalis* using GEE.

Treatment with both CS and K-Y Jelly appeared to decrease the proportion of women with PMN on Gram stain. In order to take into account baseline variations in the microflora, planned analyses were repeated by replacing raw values on Visits 3 and 4 with their respective change from baseline (the "change score"). Overall, there were no differences in alterations in H_2O_2-negative lactobacilli, *Gardnerella vaginalis*, *Enterococcus*, anaerobic gram-negative rods, *Mycoplasma hominis* or group B strepto-cocci comparing CS and K-Y Jelly (Fig. 1). Women in both treatment groups had decreased levels of H_2O_2-positive lactobacilli. Participants in the CS group had statistically significantly increased odds for *Escherichia coli* and *Staphyloccocus aureus* and had decreased odds for *Ureaplasma* compared to those in the K-Y group (Fig. 1).

3.3. Cytokine evaluation

Of the 40 participants in whom cytokines were measured, 5 of 21 (24%) exposed to CS gel and 9 of 19 (47%) exposed to K-Y Jelly had moderate or high levels during the study (baseline, 7 days and/or 14 days), but only two participants with moderate or high levels in each group had no elevated levels at baseline. Three participants with abnormal Nugent scores at baseline and one with an abnormal Nugent score after 1 week of K-Y Jelly use had moderate to high cytokine levels at baseline, suggesting inflammatory conditions prior to product use.

None of the cervicovaginal samples had TNF-α values above the established precision limit of detection (>22 pg/ml). Median IL-1α and IL-1β changed minimally from baseline. Median IL-8 levels decreased after product application in both cohorts. When baseline variation was not taken into account, a noticeable difference was found between the CS and K-Y groups for IL-1RA (p<.0001 on Visit 3 and p=.02 on Visit 4), which was primarily influenced by high median IL-1RA in the K-Y sexually active cohort at baseline. However, when statistical comparison was repeated on change scores, there was no such difference between treatment groups in any of the cytokines (p=.29–.98 on Visit 3; p=.37–.90 on Visit 4). In the majority of subjects, cytokine levels decreased or remained the same during product use (from baseline to 14 days) (Fig. 2).

Table 4
Comparison of product groups on primary endpoint: urogenital irritation

CS group versus K-Y group	Any evidence of urogenital irritation			Symptoms of urogenital irritation[a]			Signs of urogenital irritation		
	OR	CI	p[b]	OR	CI	p[b]	OR	CI	p[b]
After first use[c]	1.32	0.32–5.61	.8898	N/A	N/A	N/A	1.32	0.32–5.61	.8898
After 7 days[c]	1.05	0.31–3.53	1.0000	2.02	0.45–10.66	.4724	1.00	0.21–4.61	1.0000
After an additional 7 days[c]	1.74	0.50–6.48	.4856	2.16	0.39–14.42	.5116	1.21	0.30–5.06	1.0000
After all uses[d]	1.36	0.70–2.61	.3622	2.02	0.90–4.53	.0897	1.18	0.62–2.22	.6153

[a] There were no symptoms of urogenital irritation reported at 15 min after the first gel application at the study sites.

[b] Since the sample size was not based on statistical considerations, no formal hypothesis testing was performed, and the results of statistical tests should not be interpreted as formal justification for rejecting or failing to reject hypotheses, but only as supplementary information to guide clinical review. All p values are exploratory and should be interpreted cautiously in light of the relatively large number of statistical tests and low statistical power. For this reason, actual p values are reported (i.e., for the evaluation of objectives, there is no reference to an α level for Type I error) and are not adjusted for multiple comparisons. All p values are two-tailed.

[c] Exact logistical regression with center, cohort and product group as covariates.

[d] GEE analysis with center, cohort and product group as independent predictors, and with time as repeated measure.
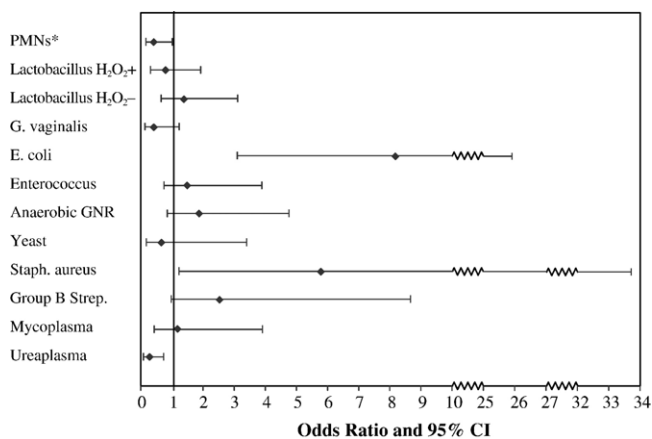
Fig. 1. Microflora and PMN: CS versus K-Y Jelly. GEE analysis comparing product groups, controlling for center and cohort, and treating time (7 and 14 days) as a repeated measure. The variable analyzed is change from baseline to follow-up. *PMN values were obtained by Gram stain.

3.4. Acceptability

About 72% of women in the CS group and 80% in the K-Y group said that they would buy the products to prevent pregnancy, and about 62% in the CS group and 70% in the K-Y group would buy the products to prevent STIs. There was no noticeable difference in the odds of the preplanned acceptability endpoint, "would not buy the product for either STI or pregnancy prevention," between the CS and K-Y groups (p<.84).

All of the women found the product either "easy" or "very easy" to use, and most had no problems inserting it. Of the 29 participants who used CS gel, 11 did not feel the gel, 13 found the gel sensation pleasant and 5 found the gel unpleasant. Of the 30 participants who used K-Y Jelly, 18 did not feel the gel, 6 found the gel sensation pleasant or very pleasant and 6 found the gel sensation unpleasant. More women in the sexually abstinent group than in the sexually active group found the sensation unpleasant.

Product leakage was the most common problem encountered with both products, and most women reported at least very slight product leakage. Of note, 7 of 16 (44%) women in the CS sexually abstinent cohort, 4 of 12 (33%) in the K-Y sexually abstinent cohort, 2 of 11 (18%) in the CS sexually active cohort and 4 of 15 (27%) in the K-Y sexually active group reported that leakage would prevent them from using the gel in the future. However, there was no noticeable difference in the proportion of overall vaginal leakage of moderate or severe intensity between the CS and K-Y groups (p=1.00).

3.5. Total AEs and systemic safety

More than four women experienced AEs in each of two body system categories: gastrointestinal disorders and reproductive system and breast disorders. There were no obvious differences between the CS and K-Y groups in proportions experiencing these types of AEs (p≥.22).

There was a total of 37 AEs in 15 (50%) participants in the CS group compared to 23 AEs in 13 (43%) participants in the K-Y group, including the urogenital AEs already described.

The most common AE that was not considered urogenital irritation was nausea, which was more common in the CS group. None of these complaints was thought to be related to the study product by the site study investigator, and none was severe. Two moderate AEs, Gardner duct cyst (CS abstinent group) and herpes simplex virus (K-Y abstinent group), were not counted toward the genital endpoint because they are not relevant to the assessment of product safety. There were three additional individuals in the CS group who reported four moderate nonurogenital AEs, including neck and back pain, nausea and headache. One male participant in each product group reported a mild AE, increased vaginal tone on the penis (which was probably product-related) and lower abdominal pain (which was considered unrelated).

There were no apparent differences between groups in mean values at follow-up in regard to laboratory parameters. There were 15 abnormal systemic safety laboratory values on the final visit among five participants in the CS group and six participants in the K-Y group. Except for two participants who had a mild inflammatory process (based on their CBC) that was associated with a viral syndrome and shifts in the vaginal flora, these abnormal values were not clinically significant.

4. Discussion

This study was conducted to assess the safety of CS when used vaginally twice daily for 14 days in a sexually abstinent cohort and in a sexually active cohort. There were slightly increased odds of experiencing any evidence of urogenital irritation in the CS group compared to the K-Y group, primarily because of increased odds of symptoms. About one third of the women in the CS group and one quarter of women in the K-Y group reported slight symptoms of urogenital irritation, which were not clinically significant but did increase the OR to an almost significant level (p<.09), even in this small study. It is unlikely that intermenstrual spotting is a product effect of CS as two of the three participants who experienced spotting had other contributing factors. Although nausea occurred more commonly in the CS group, a causal relationship is unlikely; still, future studies should continue to explore these observations.

Slightly more women in the CS group had at least one colposcopic sign of genital irritation during follow-up. In an earlier study, the only cervicovaginal findings that were associated with HIV infection were grossly observed epithelial breaches [2]. In this study, no findings of deep disruption of the epithelium were observed in either group. Seventeen percent of women in each treatment group had at least one finding of superficial disruption of the epithelium. Thus, CS does not appear to cause more epithelial disruption than K-Y.
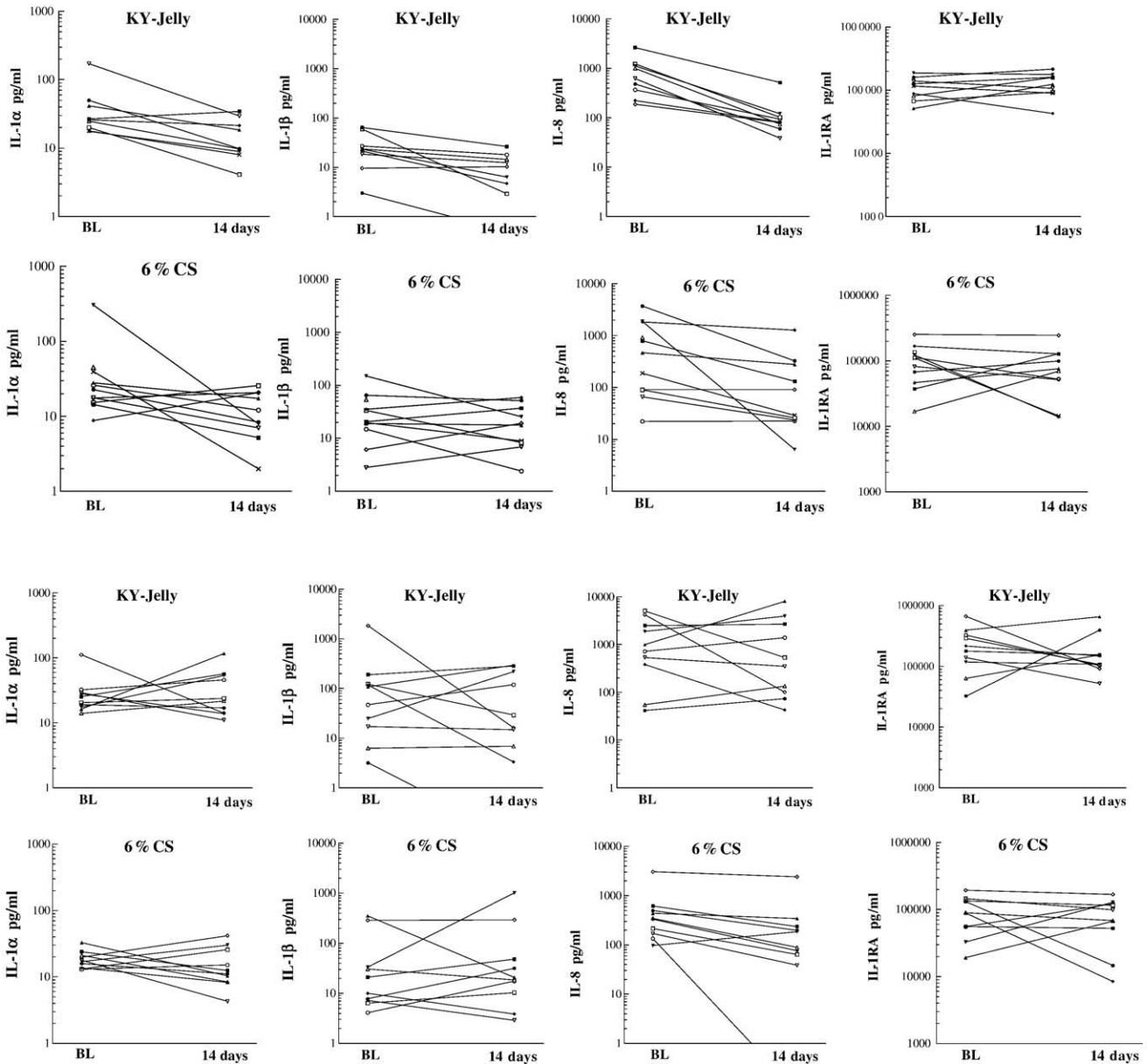
Fig. 2. Comparison of cytokine levels. Top two rows: cytokine levels of sexually abstinent cohort. Comparison of baseline values versus values obtained 14 days after product (K-Y Jelly or 6% CS gel) use. Each line represents an individual subject. Bottom two rows: cytokine levels of sexually active cohort. Comparison of baseline values versus values obtained 14 days after product (K-Y Jelly or 6% CS gel) use. Each line represents an individual subject.

At least one colposcopic finding was observed in 60% of sexually abstinent women and in 47% of sexually active women, which was somewhat reversed from an earlier observational study in which colposcopic findings were seen in 25% of women examined within 24 h of vaginal intercourse compared with 14% of women who abstained [13]. It is possible that the gel provided some type of protection in sexually active women, although other possibilities are plausible.

Ensuring that a product does not significantly disrupt the vaginal flora and does not increase urogenital infections has become standard for the evaluation of microbicide candidates. Semiquantitative vaginal cultures for the measurement of such changes have been used in previous studies with microbicide candidates, but the clinical relevance is not entirely known. The increase in *E. coli* in the CS group and the decrease in H_2O_2-positive *Lactobacillus* in both groups were the most striking shifts in the vaginal flora. Both changes have been previously reported with the use of N-9 and BufferGel [14,15], and with cervical caps and diaphragms with spermicides [16]. However, whether these differences will result in urogenital infections in a larger clinical study is unknown. The increased OR observed with *S. aureus* is predominantly due to a decrease of these bacteria in the K-Y group rather than due to an increase of these bacteria in the CS group. Furthermore, the reduction in *G. vaginalis* and *Ureaplasma* in the CS group might be potentially beneficial.

Our study is the first to report on the measurement of cervicovaginal cytokine levels to assess urogenital irritation in response to vaginal products in a clinical safety study. Cytokines regulate mucosal immune responses and, in the vaginal environment, may play a critical role in the pathogenesis of HIV-1 and other STIs [17]. N-9-induced vaginal inflammation has been linked to increased HIV activity via IL-1-mediated mechanisms [18]. A group of cytokines, some of which have been shown to be released from immortalized human vaginal epithelial cells in vitro and from rabbit vaginal mucosa after exposure to surface-active microbicide candidates [19], was evaluated to help determine their potential as an in vivo system for monitoring the undesirable proinflammatory effects of microbicides and other vaginal products. In this study, neither product appeared to have proinflammatory effects. The complicating role of high baseline cytokine levels, possibly attributable to subclinical inflammatory conditions, underscores the importance of obtaining baseline values.

Participants were more likely to buy the product for pregnancy prevention than for STI prevention, which fits the careful selection of low-risk women who perceived that they were not at risk for STIs. More women in the sexually abstinent cohort found leakage to be a problem. The purpose of testing the product in an abstinent cohort was to establish safety before progression to gel use during sexual activity. In actual practice, the gel would be used during intercourse, during which some of the gel would be distributed and leakage would be more expected and better tolerated, as noted with the sexually active women in this study.

CS was safe for twice-daily use for 14 days in sexually abstinent and sexually active women. It was associated with only slightly higher odds of reported symptoms of minor urogenital irritation. In addition, there were minor shifts in genital flora, but there was no evidence of greater inflammation as evidenced by few colposcopic findings, decreased influx of PMN and minimal changes in proinflammatory cytokines. Moreover, both products appeared acceptable to most women. CS is appropriate for future studies in effectiveness trials.

Acknowledgments

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