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Properties of a New, Long-Lasting Vaginal Delivery System (LASRS) for Contraceptive and Antimicrobial Agents

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ABSTRACT: In view of the need for improved vaginal formulations that are contraceptive, that may prevent transmission of sexually transmitted infections, or both, a new delivery system (base formulation; called Long Acting, Sustained Release of Spermicide, or LASRS) was developed that contains bioadhesive and other ingredients with a long history of safety, and was designed to provide long-lasting vaginal retention of the formulation and to minimize possible vaginal irritation caused by incorporated active ingredients. Nonoxynol-9 (N-9) was added as an active ingredient to study the vaginal irritating properties of the formulation and to assess its long-term effectiveness by postcoital spermicidal tests. In the first series of experiments, in vitro studies showed that the formulation spreads rapidly over a cellulose membrane, forming a bioadhesive layer that remained for at least 12 hours. The second series of experiments addressed the safety of the LASRS suppository in rabbits and primates. Even with a very high concentration of N-9 (20.5%), LASRS caused only mild/moderate but acceptable irritation in the rabbit. No vaginal irritation occurred in the primate at an even higher concentration (22.5%). During the third series of experiments, the long-lasting vaginal retention properties were evaluated by postcoital spermicidal tests in the primate. LASRS with N-9 was highly spermicidal even when mating was delayed for 12 hours after placement of the formulation. Spermicidal activity was also observed when 1) mating was delayed for 24 hours after insertion of the formulation, and 2) if the females were mated 2 or even 3 times without reinsertion of the suppository before collection of the vaginal contents. In the final series of tests, the postcoital spermicidal properties of menfegol, another cytotoxic spermicide, were evaluated as were several modifications in the base formulation. Menfegol produced essentially the same results as N-9. Altering the base formulation proved to be nonbeneficial because a decrease in the long-term spermicidal effectiveness was obtained. These results suggest that the LASRS suppository has good vehicle properties for the delivery of active ingredients to the vagina.

Key words: Vagina, microbicide, formulation, bioadhesion, nonoxynol-9.

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Unplanned pregnancies (United Nations, 1998) and sexually transmitted infections (STIs), including acquired immunodeficiency syndrome (AIDS), continue to be a major problem. In regard to STIs, 333 million cases of chlamydia, trichomonas, gonorrhea, and syphilis occurred worldwide in 1995 in adults between the ages of 15–49, as did 2 million new cases of human immunodeficiency virus (HIV; Elias and Coggins, 1996; Gerbase et al, 1998). For women, the greatest risk of acquiring STIs/AIDS is through heterosexual intercourse. This mode of STI transmission can be minimized through changes in sexual behavior, the use of condoms, or both. However, due to their relatively poor popularity, these methods have only minimally affected the spread of STIs/AIDS. Two other techniques can be used to prevent heterosexual infections, vaccines and vaginal ''barrier'' methods. Although a vaccine can be effective, its usefulness is somewhat limited because it is generally active only against a single STI-causing organism or strain. By contrast, a single vaginal method can, at least in theory, prevent a number of different diseases and, if desired, can also provide protection against unplanned pregnancy (Elias and Coggins, 1996; Zaneveld et al, 1996). Another benefit of vaginal methods is their control by a woman, the individual who is at greatest risk of heterosexually transmitted diseases.

Vaginal formulations consist of an active ingredient in a delivery system (''base''). The base frequently makes up about 92%–98% of the final formulation, and can have

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a major effect on the properties and activity of the active ingredient. Presently marketed vaginal contraceptive delivery systems, including gels, creams, suppositories, foams, films, and tablets, all have several limitations that may decrease the overall effectiveness of the final product. A major problem is the relatively short retention of the formulations in the vagina. For instance, of 5 products tested, including a foam, gel, film, and suppository, only from 3% (Advantage S; Columbia Laboratories, Aventura, Fla) to 19% (Conceptrol; Advanced Care Products, Raritan, NJ) is retained for 2 hours after vaginal placement (Witter et al, 1999). This short-term retention greatly decreases the protective properties of the formulations if intercourse does not take place quickly. In addition, these formulations may be displaced during the coital act. Furthermore, the short dispersion time of the presently marketed contraceptive products quickly exposes the vagina to the entire dose of active ingredient, which exacerbates the vaginal irritating effect of the most frequently used spermicide, nonoxynol-9 (N-9; Roddy et al, 1993). Vaginal irritation or lesions may enhance HIV transmission and has been cited as one of the reasons presently marketed N-9 products are not effective as anti-HIV products (Kreiss et al, 1992; Roddy et al, 1998a) and may even increase HIV transmission (Stephenson, 2000) even though N-9 is known to be a potent anti-HIV/STI agent in vitro (reviewed by Zaneveld et al, 1994).

With the renewed interest in vaginal products that prevent unplanned pregnancies, STI/AIDS transmission, or both, the development of improved base formulations for the delivery of active ingredients is receiving some attention (Rencher, 1998; Zaneveld et al, 2001). Some time ago, a new base composition was patented (Ahmad et al, 1991), consisting of a polymeric gum, a dispersing agent, and a water-miscible polyethylene glycol polymer (Chiou and Riegelman, 1971; Kellaway and Marriott, 1975; Yang et al, 1979; Steizer and Klug, 1980; Callahan et al, 1982; Kelco Technical Bulletins, 1985, 1988). The polymeric gum (carboxymethylcellulose and xanthan gum) aims at providing viscous adhesive qualities to the mixture. The dispersing agent (colloidal silicone dioxide) contributes homogeneous melt and spread characteristics to the mixture and acts to absorb and hold the spermicidal agent. It was hoped that this formulation would form a bioadhesive, slow-dissolving layer over the vaginal surface that would be retained for prolonged periods of time. In addition, it was hoped that the layer would trap the active ingredient and release it slowly so that the vagina would not be immediately exposed to the entire dose of active ingredient, thereby minimizing its possible toxic effects on the vaginal epithelium.

In order to determine if the desired properties were obtained, a base formulation containing these components, called Long Acting, Sustained Release of Spermicide (LASRS) was manufactured in suppository form. For the present experimentation, the in vitro spreading and film-forming properties were determined in the first series of tests. Subsequently, N-9 was incorporated at a very high concentration (20.5% and 22.5%), and another series of experiments were performed to determine the vaginal safety of the product in the rabbit and primate. N-9 is highly cytotoxic and can be expected to cause major damage to the vaginal epithelium at these doses unless the formulation provides some protection. In a third series of experiments, the vaginal longevity under mating conditions of the LASRS suppository was evaluated through primate postcoital spermicidal assays. Because such tests utilize sperm motility as an outcome, a sperm immobilizing agent needed to be incorporated in the formulation. Therefore, N-9 was again incorporated in the formulation. In a final series of tests, studies were performed to assess if the longevity of action of LASRS was indeed due to the formulation and not to the N-9. Menfegol, another spermicide, was incorporated into LASRS instead of N-9, and the product was evaluated in primate postcoital tests. Menfegol is a branched chain surfactant (N-9 has a straight chain) and is marketed as a contraceptive in tablet form in Japan (Neo-Sampoon; Eisai Company, Tokyo). As part of this final series of experiments, changes were made in the composition of the suppository and a LASRS gel was also prepared to determine if these alterations would enhance the long-term effectiveness of LASRS as assessed by primate postcoital tests.

The present report describes the results of these preclinical studies, which suggest that the desired properties of the base formulation were obtained. A clinical trial with the LASRS suppository was recently completed (Ladipo et al, 2000), which further confirms these characteristics (see ''Discussion''). LASRS appears to provide a good base for the incorporation and vaginal delivery of contraceptive and anti-STI agents. Ideally, such active ingredients are not or are only minimally cytotoxic (Zaneveld et al, 1996), but cytotoxic agents can also be considered.

Materials and Methods

Materials

LASRS suppositories were manufactured by Advanced Care Products (North Brunswick, NJ), containing 0% or 10%–25% N-9. Other suppositories were manufactured with the same composition but contained 10%–25% menfegol. N-9 suppositories were also produced containing 0.5% less colloidal silicone or 0.5% less colloidal silicone and 0.5% less carboxymethylcellulose while keeping the other components at the same concentration. Finally, N-9 gel and menfegol gel formulations were prepared by increasing the amount of propylene glycol while retaining the concentrations of the other components.

In Vitro Studies

The liquefaction rate of the LASRS suppository was tested with a glass apparatus containing a dialysis bag in a 37°C water bath. The suppository was placed inside the bag and observed. The time for complete liquefaction was recorded.

The coating action of LASRS was assessed in vitro using an apparatus simulating the vagina. Cellophane tubing was stretched inside a hollow glass jacket with water inlet and outlet tubes. The bottom and top ends of the tubing tightly surrounded the ends of the glass jacket. The formulation was placed as a bolus in the middle of one side of the cellophane tubing. When water (at 37°C) entered into the space between the cellophane tubing and the jacket of the apparatus, the pressure forced the inner surfaces of the cellophane membrane to collapse on each other. The spreading action of the formulation could be observed through the glass wall of the jacket. After reduction of the water pressure, the membrane tubing opened up and the adhesive properties of the formulation could be evaluated.

Animal Safety/Toxicity Studies

All the animal studies followed guidelines for the care and use of animals approved by the local institutions.

Vaginal Irritation: Rabbit—The purpose of this study was to evaluate the potential for vaginal irritation of LASRS with 20.5% (205 mg/mL) N-9, LASRS placebo (no spermicide), and a Conceptrol contraceptive insert with 8.34% N-9 (83.4 mg/mL; Advanced Care Products), administered vaginally every day for 10 days. A standard procedure and scoring system was used to assess the irritating properties of the formulation in the rabbit model (Eckstein et al, 1969). Briefly, New Zealand White rabbits, approximately 5 months of age, weighing between 2.6–3.2 kg, were observed daily during a 10-day quarantine period to ensure their suitability as test animals. They were singly housed in suspended, stainless steel, wire mesh cages in a room in which temperature was maintained at 17°C (± 3°C) with a relative humidity between 30% and 70%. A 12-hour light/dark cycle was maintained. The rabbits were randomly assigned to the control and test groups. Six animals were used in each test group. Daily, 1 mL of melted suppository was inserted deep into the vagina with a 5-mL syringe attached to a number 14 French catheter, lubricated with K-Y jelly (Advanced Care Products). Dosing continued for 10 days. The animals were observed daily for clinical signs of toxicity, and the vaginal openings were carefully examined for signs of erythema, edema, and discharge. All animals were weighed prior to dose administration on each day. On day 11, the rabbits were killed with Euthobarb (Schering-Plough Animal Health Care, Union, NJ) and were necropsied. The vagina of each animal was dissected out, cut longitudinally, observed for signs of gross pathology, and fixed in 10% buffered formalin. The fixed tissues were transferred to a histopathology laboratory for processing. Anterior, medial, and posterior sections from the abdominal vagina were blocked, sectioned, and stained with hematoxylin and eosin. The tissues were evaluated by Samuel W. Thompson & Associates (Sayer, PA) for the presence and severity of 1) epithelial ulceration, 2) vascular congestion, 3) leukocyte infiltration, and 4) edema. The severity of the changes in each category were scored from 0 to 4 and the scores were totaled. The scores for each vaginal section were totaled and divided by 3 for each rabbit. Based on the overall mean score, the effect of the formulation was evaluated according to the system described by Eckstein et al (1969), where a mean of <4 represents minimal irritation, 5–8 represents mild irritation, 9–11 represents moderate irritation, and 12–16 represents marked irritation.

Vaginal Irritation: Primate—Mature female stumptailed macaques (*Macaca arctoides*), ranging in weight from 6–15 kg, aged 9–23 years, and housed at the Biological Resources Laboratory (University of Illinois at Chicago, Chicago, Ill), were used for this study. No quarantine period was required because all animals were in the colony for at least 9 months prior to the study. Twelve primates were randomly assigned to 3 treatment groups using a stratified design blocked by age. The animals were treated with either LASRS containing 22.5% (225 mg/mL) N-9, LASRS placebo, or Conceptrol Contraceptive Insert containing 8.34% (83.4 mg/mL) N-9 (Advanced Care Products). The same inserting device and method of formulation application was used as described in the ''Postcoital Sperm Motility Studies in the Primate'' section. A staggered chart for dosing was used to assure adequate time for evaluation of each animal at the end of the study. All animals were dosed with a 1-mL suppository for 10 consecutive days. Each female was treated by grasping her arms and gently bending her over to provide access to the vestibule of the vagina. A close visual observation of the vaginal orifice for redness, swelling, excessive secretions, or any other external signs of vaginal irritation was then performed. Subsequently, a syringe loaded with formulation was gently inserted deep into the vagina and the substance was administered slowly while the syringe was simultaneously withdrawn. The animal was held in this position for approximately 2 minutes and then returned to her cage.

On the first day of dosing, each of the animals was anesthetized with ketamine, weighed, and a speculum was inserted in the vagina to facilitate a close examination of the interior of the vaginal vault and cervix for any signs of irritation or abnormal conditions prior to administration of the formulation. This procedure was repeated on the fifth day of dosing and on the day of biopsy. Each animal was observed daily just prior to the administration of the formulation for clinical signs. In addition, each animal was observed approximately 2 hours postdosing for clinical signs of toxicity and for the presence of suppository in the cage.

Vaginal biopsies were performed on the 11th day after administration of the first dose. A Witner biopsy instrument was used. Each animal was anesthetized with ketamine and placed on a surgical table, face down with the pelvis elevated to permit ready access to the vaginal vault. The instrument was gently inserted, and the location of the biopsy instrument was established by rectal palpation just prior to obtaining the biopsy. Each biopsy specimen was placed in a cassette and immediately immersed in buffered formalin. Biopsies were obtained from the anterior portion of the vaginal vault near the cervix, middle of the vaginal vault, and near the vaginal orifice. The samples were taken from the 3 areas in a clockwise manner to maximize the separation of the samples. The anterior biopsy was taken from about the 9 o'clock position, the middle biopsy from about the 12 o'clock position, and the exterior biopsy from about the 3

o'clock position. Immediately following the biopsy procedure, the vagina was observed for any excessive bleeding and packed with surgical sponges. Each animal received a prophylactic dose of 300 000 units/10 kg of Flocillin (Fort Dodge Animal Health, Overland Park, Kans) intramuscularly. All primates were monitored for any signs of bleeding or infection for 3 days after the biopsies were obtained.

The fixed biopsy specimens were blocked, sectioned, and stained with hematoxylin and eosin. In each of the specimens, the epithelium and the underlying stroma were evaluated separately as routinely done by Pathology Associates, Inc (Frederick, Md). The histopathological observations were divided into 5 categories: 1) inflammatory lesions (lymphocyte or mixed inflammatory infiltrate), hemorrhage, and hemosiderin; 2) the thickness of the stratum corneum; 3) nuclei of the stratum corneum; 4) thickness of the epithelium as measured from the estimated average apex of the papillae of the lamina propria that extend into the surface epithelium; and 5) rete ridge of the epithelium, referring to the prominence or depth of the epithelial rete ridges as they extend into the underlying lamina propria. Each category was assigned a score from 0 to 5 based on severity. Very minimal inflammatory infiltrates were not recorded inasmuch as a few inflammatory cells are expected normally. Also, a very minimal hemorrhage (≤2 small foci) was not recorded because it may have been induced by the biopsy procedure.

Postcoital Sperm Motility Studies in the Primate

The stumptailed macaque (*Macaca arctoides*) was selected for the postcoital studies because it is an excellent animal model for this purpose (Zaneveld et al, 1977; Zatuchni et al, 1981). The female is cooperative and easy to handle, allowing vaginal insertion of the formulation with ease and precise positioning just prior to coitus. The male mates rapidly and under controlled conditions, thereby minimizing extraneous factors (ie, those not inherent to the reproductive tract and the coital act). Mature primates were used; they were housed separately at the Biological Resources Laboratory of the University of Illinois at Chicago (Chicago, Ill). All the animals had been in the colony for at least 1 year.

The procedure involved the removal of the female from her cage, placing her on the floor, bending her over, and placing an inserting device as deep as possible into the vagina immediately adjacent to the cervix. The device was fabricated from a 3-mL plastic disposable syringe by removing the tip and flame-smoothing the barrel. One milliliter of slightly melted suppository or gel was placed in the barrel of the syringe, the plunger was replaced, and the formulation was compressed against the end of the syringe, held closed with parafilm. This amount of formulation matched the size limitations of the stumptailed macaque vagina, which averages about one-quarter the size of the human vagina (Zaneveld et al, 1977). The formulation was inserted while slowly withdrawing the inserter. The female was held in position for approximately 1 minute after insertion to assure the formulation was retained in the vagina. She was then kept in a transfer cage for about 9 minutes in order to allow adequate dispersion and spreading of the formulation. Subsequently, the female was placed in the male's cage for mating, which usually required no more than 2 minutes for completion. The animals were separated as soon as the male ejaculated.

A vaginal fluid sample was obtained as soon as possible after separation from the male by placing the female on the floor and bending her over. A 1-mL plastic tuberculin syringe containing 0.2 mL of physiological (0.9%) saline was inserted as far as possible into the vagina and the saline was expelled. The plunger of the syringe was pulled back while slowly moving the syringe caudally, thereby aspirating the vaginal fluid into the syringe. The syringe was then removed from the vagina and the total volume of recovered fluid was recorded. The saline allowed adequate fluid recovery from the vagina because the ejaculate volume of the stumptailed macaque is small, approximately 0.1 to 0.5 mL. After collection of the vaginal contents, a drop of fluid was immediately placed on a slide, and 100 randomly selected spermatozoa were studied with the aid of an ocular grid. The numbers of motile (any movement) and progressively motile (forward movement) spermatozoa were recorded. Sperm concentration was determined later using a hemocytometer. After the motility parameters were assessed, the female was douched 3 times with physiological saline (total volume approximately 150 mL), and returned to her cage. The douching procedure removed remaining spermatozoa and the seminal coagulum as well as retained formulation.

Single, Double, and Triple Mating Procedures

The single mating procedures were as follows: 1) 0-hour: the mating procedure was initiated 10 minutes after insertion of the formulation; 2) 12-hour: after insertion of the formulation, the female was returned to her cage and the mating procedure initiated 12 hours later; and 3) 24-hour: the same as the 12-hour procedure except that the mating procedure was initiated 24 hours later. During the double and triple mating procedures, the vaginal contents were collected only after the final mating. The double mating procedures were as follows: 1) 0 + 1 hour: the mating procedure was initiated 10 minutes after insertion of the formulation, the female was returned to her cage after completion of the coital act, and another mating procedure was initiated 1 hour later; and 2) 10 + 12 hour: same as the 0 + 1 hour procedure except that the mating procedures were initiated after 10 hours and 12 hours. The triple mating procedure (0 + 2 + 4 hour) was performed by initiating the mating procedure 10 minutes after insertion of the formulation, repeating the procedure 2 hours later, and repeating it once again 2 hours after the second mating.

Statistical Analyses

All statistics were performed using the Windows version of SPSS version 8.0 statistical software. Data were arcsin square-root transformed prior to parametric analysis. Analysis of variance was used to determine significance between treatment and control groups. Bonferroni procedure was employed for pairwise comparisons when a significant difference was indicated.

Results

In Vitro Studies

Complete liquefaction of the LASRS suppository occurred within 10–30 minutes, similar to that reported for

marketed suppositories. When a bolus of LASRS was placed on one side of the inner surface of hollow cellulose sulfate tubing and the tubing collapsed by water entry into the jacket of the apparatus (see ''Materials and Methods''), an even coating (''film'') formed over the inner surface of the cellophane tubing. After separating the walls of the tubing by reducing the water pressure, the formulation coating remained adherent to the walls for at least 12 hours. Similar experiments with marketed suppositories such as Intercept (Advanced Care Products) showed that these formulations also spread evenly over the tubal walls but that the coating disappeared within 30 minutes. These results indicate that LASRS spreads quickly and evenly over a membrane to form a coating with long-lasting adhesive properties.

Vaginal Irritation: Rabbit

LASRS with a high dose of N-9 (20.5%, 205 mg/mL) was selected for these studies. All animals treated for 10 days with the LASRS placebo, LASRS with N-9, and the Conceptrol suppository tolerated the vaginal dosing without difficulty except for 1 animal that had been treated with N-9 LASRS. This animal exhibited vaginal orifice erythema and edema and blood in the vaginal discharge. No other pharmacotoxic signs were observed in any of the treated animals for all groups, except for occasional vaginal discharge.

Gross observations at necropsy revealed minor erythema and edema in the groups treated with N-9 LASRS (2/6 animals) and LASRS placebo (2/6 animals). Alterations in the vaginal vault were observed in 3/5 animals treated with N-9 LASRS consisting primarily of some hemorrhagic sites. Red striations were also observed in the LASRS placebo (3/6 animals) and Conceptrol (1/6 animals) groups. Some residual material was observed in the vaginas of 1 or 2 animals for all treated groups.

Histopathological examination of the vaginas was performed blindly. An acceptable mean vaginal irritation score of 9 (borderline between mild and moderate) of a maximal score of 16 was obtained for the animals treated with 22.5% LASRS. The primary alterations were epithelial ulceration and leukocyte infiltration, with some vascular injection. A mean vaginal irritation score of 3 (minimal irritation) was obtained with the LASRS placebo. Conceptrol also scored as minimal irritation.

Vaginal Irritation: Primate

LASRS with a high dose of N-9 (22.5%, 225 mg/mL) was also tested in the primate. Administration of the material for 10 consecutive days was well-tolerated by all animals in all test groups. As in the postcoital studies, no avoidance behavior was exhibited. Some observations of residual substance resembling test material was observed within or around the vaginal orifice/opening. This was found in all dose groups with the highest frequency in animals treated with LASRS placebo.

No gross changes in the appearance of the vaginal vault were observed at either the 5- or 11-day observations. On histopathological examination, there was no difference or increased occurrence in inflammatory lesions (lymphocytic or mixed inflammatory infiltrate), hemorrhage and hemosiderin, or epithelium thickness or epithelium rete ridges in any of the treatment groups. A small decrease in the thickness and number of nuclei of the stratum corneum (keratinized layer) of only the anterior (proximal) vagina was observed in animals treated with N-9 LASRS compared with the other treatment groups. This change was not observed in the other 2 vaginal locations evaluated and was not considered significant because the epithelial thickness and prominence of the ridges were not altered.

Primate Postcoital Sperm Motility Studies

The studies were performed blindly. The code was broken only after all experimentation had been completed. Two active ingredients were evaluated, N-9 and menfegol. One milliliter of LASRS, containing a total of 100 to 250 mg/mL of active ingredient was applied to the vagina (ie, ranging from the amounts of N-9 that have been applied to the human to the high amounts found acceptable in the vaginal irritation studies). The results of the doses ranging from 100–150 mg and those from 200–250 mg were combined because no significant differences were found within these groups.

Control experiments in the absence of any formulation were performed throughout the experimentation period with the same female and male primates. The mean $\pm$ SEM (range; n) for the various assessment indices were as follows: motile spermatozoa, 62.2 $\pm$ 1.7 per 100 spermatozoa counted (range: 46–82, n = 27); progressively motile spermatozoa, 52.7 $\pm$ 2.1 per 100 motile spermatozoa (range 24–71, n = 27); sperm concentration, 149.0 $\pm$ 21.3 $\times$ 10^6/mL (range, 36–417, n = 24); and volume recovered, 0.11 $\pm$ 0.01 mL (range, 0.03–0.19; n = 18). All these indices are listed in the Tables summarizing the experimental results. No significant differences ($P > .05$) were found in regard to volume and sperm concentration between the control group and any of the experimental groups. Although the number of motile spermatozoa and, therefore, the number of progressively motile spermatozoa, varied significantly between some of the test groups and the control groups, the percentage of motile spermatozoa with forward progression tended to remain similar (ie, if spermatozoa were found to be motile, about 70%–90% possessed forward motion). Therefore, only the variations in motile sperm numbers are discussed.

Single mating results with the LASRS suppository containing N-9 are reported in Table 1. Essentially no motile

Table 1. *LASRS Suppository Containing Nonoxynol-9: Single Mating Procedures**

Matings	Nonoxynol-9, 100–150 mg			Nonoxynol-9, 200–250 mg		
	Mean ± SEM	Range	n	Mean ± SEM	Range	n
0-Hour						
Motile spermatozoa†	0.6 ± 0.6	0–7	12	0.6 ± 0.6	0–14	23
Progressive spermatozoa†	0	0	12	0.6 ± 0.6	0–14	23
Sperm concentration, 10^6 sperm per mL	201.8 ± 23.7	73–355	11	151.4 ± 33.2	31–690	23
Recovered volume, mL	0.13 ± 0.01	0.06–0.20	12	0.15 ± 0.01	0.10–0.22	23
12-Hour						
Motile spermatozoa†	9.5 ± 3.4	0–36	15	7.9 ± 3.7	0–66	23
Progressive spermatozoa†	6.8 ± 2.7	0–32	15	7.0 ± 3.4	0–62	23
Sperm concentration, 10^6 sperm per mL	147.4 ± 27.1	41–377	14	121.8 ± 10.1	43–227	23
Recovered volume, mL	0.17 ± 0.03	0.04–0.40	15	0.12 ± 0.01	0.04–0.22	23
24-Hour						
Motile spermatozoa†	ND			31.8 ± 5.1	0–58	13
Progressive spermatozoa†	ND			25.8 ± 4.7	0–50	13
Sperm concentration, 10^6 sperm per mL	ND			125.1 ± 14.0	47–210	13
Recovered volume, mL	ND			0.12 ± 0.01	0.05–0.19	13

* The following analyses relate to motile spermatozoa. All experimental conditions differ significantly ($P < .05$) from the untreated control (see text). No significant differences ($P > .05$) are present between the 0-hour and 12-hour treatment groups (independent of the nonoxynol-9 dose), but these groups differ significantly ($P < .05$) from the 24-hour treatment group. ND indicates not determined; LASRS, Long Acting Sustained Release of Spermicide.

† Number per 100 recovered spermatozoa.

spermatozoa could be found in the vagina when the mating procedures were initiated 10 minutes after placement of the suppository (0-hour) whether the lower (100–150 mg) or higher (200–250 mg) doses of N-9 were used. Even if mating was delayed by 12 hours, only a very low percentage of spermatozoa were motile and fewer possessed forward progression. No significant differences ($P < .05$) were present between the 0-hour and 12-hour results. The suppository retained spermicidal activity even if mating was delayed by 24 hours after placement of the LASRS suppository (only the higher N-doses were tested; Table 1), although this was significantly ($P < .05$) reduced compared with the 0- and 12-hour matings. These results show the LASRS suppository to be an effective vaginal spermicide whose activity is retained for prolonged periods of time, at least 12 hours.

Subsequently, the effectiveness of the LASRS suppositories with N-9 were evaluated when the females were mated 2 or 3 times without reinsertion of the formulation and the vaginal contents collected only after the last mating (Table 2). A significant ($P < .05$) reduction in motile spermatozoa compared with the controls occurred in the double mating experiments whether 1) the animals were mated 10 minutes after insertion of the suppository and again 1 hour later, or 2) 10 hours after insertion and then again 2 hours later (only the higher doses of N-9 were tested). In the first case, the higher dose of N-9 tended to be more effective than the lower dose but the difference was not significant. Finally, a significant reduction ($P < .05$) in motile spermatozoa was observed when the animals were mated 3 times before collection of the vaginal contents without reinsertion of the formulation (only the higher N-9 doses were tested; Table 2). None of the higher dose treatment regimens differed significantly from each other ($P > .05$), and only the 0 + 2 + 4-hour treatment group differed significantly from the 0-hour high dose group (Table 1). The LASRS suppository appears to retain significant spermicidal effectiveness even when mating is performed repeatedly following the single application of the formulation.

In a subsequent series of experiments, another active ingredient (menfegol) was evaluated (Table 3). This detergent proved to be as effective or tended to be slightly more effective than N-9 in the 0-hour and 12-hour single mating and the 0 + 1 hour double mating procedures. The higher doses of menfegol tended to be more effective than the lower doses in the 12-hour single mating and the 0 + 1 hour double mating procedures but the differences were not significant ($P > .05$). Thus, LASRS retains its long-term and repeat mating effectiveness when another spermicide is used as the active ingredient.

Modifications in the base composition of the suppository were made and tested. In one modification, the amount of colloidal silicone was increased by 0.5%. This change decreased the effectiveness of the LASRS suppository containing 100–150 mg/mL N-9 in the 0-hour experiments (mean sperm motility: 23.0% ± 13.0%; n = 3) as well as the 12-hour experiments (mean sperm motility: 43.3% ± 22.6%; n = 3). A decrease in both the colloidal silicone and carboxymethyl cellulose content by

Table 2. *LASRS Suppository Containing Nonoxynol-9: Multiple Mating Procedures**

Matings	Nonoxynol-9, 100–150 mg			Nonoxynol-9, 200–250 mg		
	Mean ± SEM	Range	n	Mean ± SEM	Range	n
0 + 1-Hour						
Motile spermatozoa†	28.6 ± 10.1	0–74	9	12.3 ± 3.8	0–52	15
Progressive spermatozoa†	24.9 ± 9.1	0–71	9	10.6 ± 3.6	0–47	15
Sperm concentration, 10^6 sperm per mL	141.7 ± 13.5	79–203	9	132.5 ± 17.2	64–283	15
Recovered volume, mL	0.12 ± 0.02	0.05–0.28	9	0.14 ± 0.01	0.05–0.20	15
10 + 12-Hour						
Motile spermatozoa†	ND			22.8 ± 4.5	5–50	9
Progressive spermatozoa†	ND			17.7 ± 3.2	2–25	9
Sperm concentration, 10^6 sperm per mL	ND			150.2 ± 40.6	30–450	9
Recovered volume, mL	ND			0.11 ± 0.02	0.03–0.18	9
0 + 2 + 4-Hour						
Motile spermatozoa†	ND			26.5 ± 6.2	0–46	8
Progressive spermatozoa†	ND			22.7 ± 5.3	0–39	8
Sperm concentration, 10^6 sperm per mL	ND			165.6 ± 19.8	98–261	8
Recovered volume, mL	ND			0.13 ± 0.02	0.06–0.19	8

* The following analyses relate to motile spermatozoa. All treatment groups differ significantly ($P < .05$) from the untreated control group (see text). No significant differences ($P > .05$) are present among the 4 treatment groups in this table. The 0 + 1-hour lower dose nonoxynol-9 differs significantly from the 0-hour but not the 12-hour lower dose nonoxynol-9 treatment groups (Table 1). The 0 + 2 + 4 group, but not the 0 + 1 and the 10 + 1 higher dose nonoxynol-9 groups, differ significantly from the 0-hour higher dose group (Table 1). ND indicates not determined; LASRS, Long Acting Sustained Release of Spermicide.

† Number per 100 recovered spermatozoa.

0.5% did not change the effectiveness of the formulation (100–150 mg/mL N-9) in the 0-hour experiments but caused a reduction in the 12-hour experiments (mean sperm motility, 16.4% ± 5.9%; n = 6). Thus, these modifications did not enhance the longevity of LASRS action.

A gel formulation was also tested containing the same composition as the suppository, but with additional propylene glycol. Only the lower N-9 and menfegol doses were evaluated because these tended to be most variable (Table 4). The gels containing N-9 and menfegol caused the complete or almost-complete absence of motile spermatozoa when the mating procedure was initiated 10 min-

Table 3. *LASRS Suppository Containing Menfegol: Single and Double Mating Procedures**

Matings	Menfegol, 100–150 mg			Menfegol, 200–250 mg		
	Mean ± SEM	Range	n	Mean ± SEM	Range	n
0-Hour						
Motile spermatozoa†	0	0	12	0	0	6
Progressive spermatozoa†	0	0	12	0	0	6
Sperm concentration, 10^6 sperm per mL	290.8 ± 53.5	58–627	12	214.0 ± 49.2	78–417	6
Recovered volume, mL	0.21 ± 0.04	0.04–0.52	12	0.13 ± 0.03	0.05–0.25	6
12-Hour						
Motile spermatozoa†	8.8 ± 4.1	0–46	12	0	0	6
Progressive spermatozoa†	5.2 ± 2.4	0–23	12	0	0	6
Sperm concentration, 10^6 sperm per mL	126.8 ± 14.2	27–187	12	75.5 ± 27.6	10–112	6
Recovered volume, mL	0.15 ± 0.04	0.02–0.50	12	0.15 ± 0.02	0.09 ± 0.20	6
0 + 1-Hour						
Motile spermatozoa†	24.7 ± 8.69	0–72	9	3.3 ± 1.6	0–10	6
Progressive spermatozoa†	20.4 ± 8.9	0–71	9	0.3 ± 0.3	0–2	6
Sperm concentration, 10^6 sperm per mL	114.8 ± 12.8	72–191	9	112.7 ± 11.6	78–156	6
Recovered volume, mL	0.20 ± 0.05	0.02–0.46	9	0.22 ± 0.05	0.09–0.40	6

* The following analyses relate to motile spermatozoa. All groups differ significantly ($P < .05$) from the untreated control (see text). The 0-hour, 12-hour, and 0 + 1-hour menfegol treatment groups do not differ significantly ($P < .05$) from their respective nonoxynol-9 treatment groups (Tables 1 and 2). LASRS indicates Long Acting Sustained Release of Spermicide.

† Number per 100 recovered spermatozoa.

Table 4. *LASRS Gel Containing Nonoxynol-9 or Menfegol: Single mating procedures**

Matings	Nonoxynol-9, 100–150 mg			Menfegol, 100–150 mg		
	Mean ± SEM	Range	n	Mean ± SEM	Range	n
0-Hour						
Motile spermatozoa†	0	0	3	2.0 ± 2.0	0–12	6
Progressive spermatozoa†	0	0	3	1.1 ± 1.1	0–7	6
Sperm concentration, 10^6 sperm per mL	131.7 ± 38.4	64–197	3	193.8 ± 52.0	53–353	6
Recovered volume, mL	0.11 ± 0.01	0.08–0.13	3	0.12 ± 0.05	0.02 ± 0.35	6
12-Hour						
Motile spermatozoa†	48.7 ± 6.4	36–56	3	20.2 ± 9.6	0–58	6
Progressive spermatozoa†	42.5 ± 6.4	32–54	3	12.4 ± 8.1	10–50	6
Sperm concentration, 10^6 sperm per mL	94.3 ± 16.0	71–125	3	221.7 ± 64.7	40–413	6
Recovered volume, mL	0.2 ± 0.11	0.07–0.43	3	0.15–0.02	0.07–0.23	6

* The following analyses relate to motile spermatozoa. All treatment groups except for the 12-hour nonoxynol-9 group differ significantly ($P < .05$) from the untreated control group (see text). The 0-hour treatment groups do not differ significantly ($P > .05$) from their respective 0-hour suppository groups (Tables 1 and 3). Significant differences ($P < .05$) exist between the 12-hour nonoxynol-9 group but not the 12-hour menfegol group and their respective suppository groups at the same time period (Tables 1 and 3).

† Number per 100 recovered spermatozoa.

utes after insertion. However, the longevity of the N-9 gel was significantly reduced ($P < .05$) compared with the standard suppository (Table 1) when the mating procedure was delayed by 12 hours. Although the menfegol gel also tended to have lower spermicidal activity if mating was delayed by 12 hours, this was not significantly different ($P > .05$) from that seen with the 100–150 mg menfegol suppositories (Table 3). It was concluded that the LASRS gel provided no benefits over the suppository.

Discussion

Presently marketed vaginal contraceptive formulations are an important weapon in our armament against unplanned pregnancies. In addition, they afford some protection against gonorrhea and chlamydia infections transmitted through heterosexual intercourse (Roddy et al, 1998b), although not against the transmission of HIV (Kreiss et al, 1992; Roddy et al, 1998a; Stephenson, 2000). However, they do not remain in the vagina for prolonged periods of time, requiring intercourse to take place rapidly after placement, which reduces consumer satisfaction and use-effectiveness. For instance, according to the package inserts, K-Y Plus and Conceptrol (both marketed by Advanced Care Products, Raritan, NJ) are effective for only 1 hour or less. Clinical trials with 5 marketed products showed that up to 96% of the formulations are removed from the vagina within 2 hours (Witter et al, 1999). In addition, such marketed formulations have never been reported to produce a bioadhesive layer over the human vaginal and cervical surfaces, which is visible with a colposcope. This layer was also not found in clinical studies with Conceptrol (Ladipo et al, 2000).

Using a cellophane membrane, in vitro studies showed the LASRS suppository to have good spreading and adhesive properties, and to form a layer over the membrane that was retained for at least 12 hours. The rabbit vaginal irritation studies, performed by applying 1 ml LASRS containing 205 mg N-9 for 10 consecutive days, produced an overall score of 9, which falls between the borderline of mild and moderate irritation, and is considered acceptable for initiation of a human clinical trial. Although the rabbit vaginal irritation model is widely accepted as the standard for assessment of potential toxicity of vaginal products, its vaginal epithelium and its neutral pH differ greatly from the stratified squamous epithelium of the human vagina and its acidic pH (3.5–4.5). Therefore, results obtained in the rabbit may not reflect those in the human, so additional tests were performed in the primate. In this species, LASRS with an even higher concentration of N-9 (225 mg/mL) was used, applying 1 mL for 10 consecutive days. No vaginal irritation occurred. The greater sensitivity of the rabbit vagina compared to the primate vagina for N-9 products has been reported previously (Eckstein et al, 1969). We speculate that the good safety profile of LASRS is due to entrapment of the N-9 in the long-lasting surface film and its slow dispersion in relatively small quantities. After its dispersion, N-9 is fairly rapidly removed from the vagina, probably primarily through leakage. Therefore, the vaginal epithelium is exposed only to relatively low concentrations of N-9 over time, in contrast to presently marketed contraceptive products that dissolve quickly in their entirety, exposing the vagina to a high concentration of N-9 in a short period of time.

After incorporation of N-9, the longevity of action of the LASRS suppository was tested in primate postcoital

tests. One-milliliter formulation was applied to the primate vagina before mating either once, twice, or three times with collection of the vaginal contents only after the last mating. The lower dose range of N-9 (100–150 mg) has been used in women. The higher dose range (200–250 mg) is the amount found to be acceptable in the animal vaginal irritation studies (see above). The LASRS suppository immobilized essentially all spermatozoa consistently when the mating procedure was initiated 10 minutes after formulation insertion, independent of the dose of N-9 tested. The occasional motile spermatozoa normally had no forward progressive movement. These results compare well with those obtained previously in the same primate model with some marketed contraceptive preparations (Zatuchni et al, 1981). For instance, 1 mL of Delfen Contraceptive Cream (Ortho Pharmaceuticals Company, Raritan, NJ), containing a total of 50 mg N-9, averaged a sperm motility of 17% ± 5%. The same amount of Delfen Contraceptive Foam (Ortho Pharmaceuticals), containing a total of 125 mg N-9, averaged 4% ± 3% motile spermatozoa.

The LASRS suppository retained its spermicidal efficacy for prolonged periods of time. Even if mating of the primates was delayed for 12 hours, only a small percentage of the recovered spermatozoa were motile and an even smaller percentage showed forward progressive movements. The differences between the 0-hour and 12-hour results were not statistically significant, nor were the differences between the higher and lower doses of N-9. Some spermicidal effectiveness was retained for at least 24 hours. These long-term spermicidal results are even more impressive considering the females were free in their cages during the waiting period and at times climbed around quite vigorously. High spermicidal effectiveness was not observed with Conceptrol (Advanced Care Products; a marketed vaginal contraceptive) when mating was delayed for 12 hours, producing a mean sperm motility of 31% (data not presented). The long-term effectiveness of the formulation is most likely due to the formation of a surface film that is retained for prolonged periods of time, gradually dispersing the N-9.

A significant decrease in the percentage of recovered motile and progressively motile spermatozoa (compared with controls) was also obtained when the primates were mated twice before collection of the vaginal contents whether the first mating occurred 10 minutes or 10 hours after insertion of the suppository and repeated 1 or 2 hours later without reinsertion of the formulation. Under these repeat mating conditions, the higher N-9 doses tended to be more effective than the lower concentrations. The higher N-9 levels even retained spermicidal efficacy when the animals were mated 3 times in a 4-hour period without reinsertion of the formulation before collection of the vaginal contents (the lower N-9 doses were not tested). These results show that at least some of the formulation remained in the vagina during penile manipulation and in the presence of semen.

In order to determine if the long-term spermicidal potency of LASRS suppository is retained with a different active ingredient, menfegol was substituted for N-9, and the 0-hour and 12-hour mating experiments were repeated. The menfegol suppository proved to be at least as active as the N-9 suppository. As with N-9, no significant differences were found between the lower and higher doses of menfegol. The menfegol suppository was also at least as effective as the N-9 suppository when double-mating experiments were performed without reintroduction of the formulation. Similar to LASRS with N-9, a significant difference between the suppositories containing lower and higher doses of menfegol, was only noted in the repeat mating experiments. These observations suggest that the LASRS suppository retains efficacy over long periods of time and also on repeat mating, independent of the spermicidal ingredient.

Relatively small changes in the composition of the LASRS suppository can cause a major decrease in its activity, particularly when mating is delayed for 12 hours. For instance, a 0.5% increase in the concentration of colloidal silicone (from 2.5% to 3.0%) in the suppository caused a large reduction in efficacy of the lower-dose N-9 suppositories. Mean sperm motilities of 23% and 43% were obtained, respectively, for the 0-hour and 12-hour mating experiments (compared with 0.6% and 9.5% motility for the standard 100–150 mg N-9 suppositories). Although the 0-hour efficacy was retained when the formulation was changed to a gel containing 100–150 mg/mL N-9 or menfegol, the 12-hour effectiveness was reduced. These results further support the contention that the vehicle, rather than the N-9, causes the long-lasting retention of the formulation.

After the present studies were completed, human clinical trials were performed with the LASRS suppository (Ladipo et al, 2000). The occurrence of a long-lasting bioadhesive layer over the vaginal and cervical surfaces was confirmed. This layer was quite tightly attached and could not be removed by rinsing the human vagina with saline or an acetic acid solution, and remained in place for 12 hours or more. These clinical trials also showed that LASRS with 20% N-9 caused no vaginal irritation, as judged by colposcopy, when applied once or for 7 consecutive days to the vagina. Because our present studies showed that LASRS with 20.5%–22.5% N-9 caused mild to moderate vaginal irritation in the rabbit but no irritation in the primate, these results suggest that the primate may be a more appropriate animal model for vaginal irritation studies for data being extrapolated to women. Finally, postcoital tests in women with LASRS + N-9 confirmed its long-term effectiveness. Motile spermatozoa were vir-

tually absent in cervical mucus even after delaying intercourse from 5–8 hours after placement of the LASRS suppository.

Based on these observations, it can be concluded that the LASRS formulation forms a long-lasting, bioadhesive, surface film. It is anticipated that this film will help protect against contact of STI-causing microbes with the vaginal epithelium. LASRS has long-term retention in the vagina, most likely because the surface film is dispersed slowly. Our results also suggest that an incorporated active ingredient is released gradually, probably by being trapped in the surface film, providing protective activity for prolonged periods of time. Such slow dispersion may expose the vagina to only relatively low concentrations of the active ingredient over time, minimizing its vaginal irritating potential. LASRS is worth considering as a vehicle for the new active ingredients that are presently under development (Elias and Coggins, 1996; Pauwels and De Clercq, 1996; Zaneveld et al 1996; Stone, 1997).

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