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Formulation and evaluation of metronidazole acid gel for vaginal contraception

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Abstract

In this study, the efficacy of metronidazole as a local spermicidal agent was investigated. The drug was formulated in a concentration of 5% as an acid gel for vaginal application as a local contraception. The minimum spermicidal concentration of metronidazole was evaluated in-vitro and found to be 0.2% w/v. The formulated gel showed instant immobilization and death of all sperm within 30 s. A clinical study was conducted to determine the drug concentrations in vaginal secretions of healthy women and women with symptoms suggesting genital tract infection every 15 min for a 1-h period after application of 1 g of the gel in the vagina. Drug concentrations in the infected group were significantly lower ($P < 0.05$) than those in the healthy group at all time intervals. The drug concentrations in vaginal secretions after 1 h of vaginal medication remained above the determined minimum spermicidal concentration (0.2%) in the two groups. Measurement of vaginal pH before and after medication revealed a significant drop ($P < 0.05$) in pH to its normal value in both healthy and infected groups as a result of gel application. In conclusion, the designed gel has potential advantages of achieving a long retention time and effective drug concentrations in the vagina for at least 1 h after application, and of maintaining vaginal pH within its normal range.

Introduction

Vaginal contraception has great health benefits due to its prophylactic effect on the spread of venereal disease and its prophylactic control of other vaginal infections. The vaginal formulations are easy to use without advance planning, can provide extra lubrication during sexual intercourse and reduce the incidence of side-effects resulting from systemic treatment. Several spermicidal products have been entered into human trials and many of them are classified as unsafe by the Food and Drug Administration. This reflects a real need to develop safe spermicidal agents and products.

Metronidazole (1-(2-hydroxyethyl)-2-methylnitroimidazole) has proved to be very effective for the therapy of amoebiasis, trichomoniasis, lambliasis and anaerobic infections (Tracy & Webster 1996). The drug is well tolerated and widely used in clinical practice in the form of solutions, tablets and vaginal formulations. The efficacy of metronidazole in the treatment of Trichomonas vaginalis has been well documented (Durel et al 1960; Spence et al 1997). Metronidazole gel is currently used to treat bacterial vaginosis, since it is easy to apply and has fewer side-effects compared with oral forms of the medication (Livengood et al 1994; Hanson et al 2000).

Metronidazole has been shown to be mutagenic in bacterial assay (Voogd 1981). The mutagenic effect of metronidazole in bacteria is thought to be based on the reduction of the nitro group to a reactive intermediate that attacks microbial DNA. This inhibits further DNA synthesis and causes degradation of already existing DNA (Knight et al 1978). This mutagenic activity may also be responsible for some reproductive toxicity of metronidazole, including the inhibition of spermatogenesis in rats. McClain et al (1989) conducted a dose–response study in male rats to quantitatively assess the effects of metronidazole on male fertility, reproductive organ weights, testicular and epididymal sperm counts, epididymal sperm viability and morphology, and histopathology of the reproductive tract. They reported that at a high oral dose of 400 mg kg^{-1} per day for 8 weeks, metronidazole produced infertility in the male rats

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through inhibition of spermatogenesis as early as the stage of the primary spermatocyte. These effects of metronidazole drew attention to the importance of investigating its spermicidal activity.

Intravaginal metronidazole is effective, safe, well tolerated and already established treatment for bacterial vaginosis. The efficacy of intravaginal metronidazole does not appear to be affected by exposure to semen (Livengood et al 1994). It was not proved that metronidazole has an effect on genetic make-up and development of the foetus. These may be good reasons to enter metronidazole into human trials as a spermicidal agent.

Very few studies have been conducted concerning the efficacy of metronidazole as a local spermicide. Safwat et al (1988) investigated the spermicidal effects of a variety of chemical compounds and their preparations. They reported that 1% w/v metronidazole in carbopol hydrogel was enough to instantly immobilize human sperm in-vitro. Omar (1995) found that the minimum inhibitory concentration of metronidazole as a spermicide in solution was 0.2% w/v, which is very high compared with that of 1–50 μg mL^{-1} for the drug as a bactericide (Tracy & Webster 1996). Omar (1995) studied the spermicidal activity of different concentrations of metronidazole dispersed in plastibase ointment formulated with amberlite IRP-69 ion exchange resin. The results showed a minimum drug concentration of 20% that induces in-vitro spermicidal action on human sperm within 4 min. This concentration of the drug in the ointment base is very high compared with its minimum inhibitory concentration as a spermicide, reflecting the effect of the drug formulation on the drug activity. This may be explained by the low effective drug concentration in the formulated ointment bases due to the presentation of the drug mainly in a dispersed rather than a soluble form. These results reflect the need to optimize the drug formulation to achieve a high effective drug concentration.

This study was conducted to formulate metronidazole in a suitable form for vaginal contraception taking in consideration two important points. The first point is the enhancement of drug solubility in the formulated product to achieve a high effective drug concentration. The second point is the optimization of the spermicidal activity of the drug in the formulated product.

Materials and Methods

Materials

The materials used were metronidazole (Rhône-Poulenc Santé, Vitry-sur-Seine, France), methyl cellulose (Sigma, St Louis, MO, USA), and glacial acetic acid (Merck, Darmstadt, Germany). All other chemicals used were of analytical grade.

Formulation of metronidazole acid gel

Metronidazole gel was prepared using the following formula: metronidazole (5% w/w), glacial acetic acid (25% w/w) and 3% w/w methyl cellulose gel (70% w/w). Methyl cellulose gel was prepared by dispersing 3.0 g methyl cellulose powder in 50 mL hot water (70 °C). The mixture was stirred for 10 min until a homogenous dispersion was obtained. A suitable volume of cold water was added to the previous mixture with continuous stirring to achieve the required weight. The product was refrigerated for 1 h before use. The metronidazole acid gel was prepared by dissolving the drug in glacial acetic acid and then adding the solution to the prepared methyl cellulose gel.

Rheological study

The rheological properties of the prepared gels were measured using a Brookfield Digital Rheometer (Model DV-III, with a Helipath spindle C; Brookfield Engineering Laboratory, Stoughton, MA, USA). The system was managed by Brookfield Rheocalc software (version 1.3). The tested samples were placebo gel, acid gel and metronidazole acid gel. The consistency of the tested systems was measured at 20 °C and a range of rates of shear (17.4–121.5 s^{-1}) (speed range of 20–140 rev min^{-1}). The speed of the spindle was adjusted to be increased by a value of 20 rev min^{-1} every 5 min and then reduced by the same pattern when the maximum speed was reached. The time of 5 min was used to allow for homogenous distribution of the shearing force throughout the sample before readings were taken.

In-vitro evaluation of the spermicidal activity of metronidazole

The spermicidal activity of metronidazole was evaluated according to the International Planned Parenthood Federation's Agreed Test for Total Spermicidal Power (Kleinman 1964). Human semen was collected by masturbation into a clean, dry, sterile wide-mouthed glass jar. The semen must be less than 4 h old, have a sperm count of greater than 50 million sperm mL^{-1}, have at least 40–50% fully motile sperm on initial examination and appear suitably liquefied to ensure proper measurement and mixing. The semen samples were incubated at 37 °C until use.

The Agreed Test for Total Spermicidal Power was performed by mixing 0.2 mL fresh semen with 1.0 g of placebo gel (free from drug and acid) and 11.0 mL normal saline containing different concentrations of metronidazole (0.0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6% w/v). The mixture was mixed thoroughly at 37 °C and a sample of 10 μL was transferred to a clean slide and immediately examined microscopically. Motility of sperm was recorded at 0, 0.5, 1, 2, 6, 10, 15, 20, 25 and 30 min, and the time of complete immobilization was recorded. Viability of sperm was evaluated at the same time intervals using the vital stain method reported by Safwat et al (1988). A sample of 10 μL of the previously prepared mixture was mixed with 20 μL of eosin stain (0.5% w/v) on a dry, clean slide. The mixture was spread into a thin film, left to dry and examined microscopically. The dead sperm took the red stain, while the living sperm remained colourless. The percentage viability was recorded

by counting the stained sperm at the specified time intervals. According to International Planned Parenthood Federation, the minimum acceptable spermicidal concentration is the concentration that showed complete immobilization of all sperm at 40 s, and death of all sperm within 30 min of incubation with alkaline solution (Kleinman 1964).

In-vitro evaluation of the spermicidal activity of the gel products

The spermicidal activity of the acid gel (25% acid) and the metronidazole acid gel (5% drug and 25% acid) were determined by mixing 0.2 mL fresh semen with 1.0 g of the tested gel and 11.0 mL normal saline. The test was performed using the same procedure described for the evaluation of metronidazole spermicidal activity.

Clinical study

This study was conducted to ensure the clinical efficacy of the prepared metronidazole acid gel as a spermicide. The study was designed to evaluate the drug concentrations in vaginal secretions of women after vaginal application of the gel.

Study population

The study was conducted at the out-patient clinic of the Department of Obstetrics and Gynecology, Tanta University Hospital between June 1999 and January 2000. The patients enrolled in the study had an average age of 30 years (range 25–45 years). The study protocol was approved by the institutional ethical committee for the use of humans in research. This committee comprises three full professors at Tanta University Hospital. The details of the study were described to the patient and written informed consent was obtained before the start of study. The patients were categorized into two groups, with 48 subjects in each group. Group 1 included those who were healthy and free from genital tract infection; group 2 included those with symptoms suggesting genital tract infection. These symptoms included offensive vaginal discharge, frequency and urgency of micturition associated with burning sensation, itching or dysuria, lower abdominal pain especially on one or both iliac fossa, or backache. A thorough medical history was taken and any pregnant women, patients with a missed period, and those with vaginal bleeding (either menstruation or abnormal bleeding from genital tract) were excluded from this study.

Collection of vaginal secretions

Each patient was prepared in the lithotomy position and a volume of 5 mL normal saline was instilled through a graduated plastic dropper into the posterior fornix. The saline was thoroughly mixed with vaginal secretions by repeated withdrawal and injection of the fluids. The contents were then aspirated using a sterile plastic dropper, transferred to Eppendorf tubes and stored at $-20\,^{\circ}C$ until analysis. This sample was used as a blank to construct the calibration curve. The medicated acid gel (1 g, equivalent to 50 mg metronidazole) was aspirated from its container using a tuberculin syringe and instilled into the posterior fornix; the patient, in the dorsal position, adducted her flexed hips and knees toward the abdomen to avoid leakage of the medicine from vagina. The patient was kept in this position for at least 5 min to avoid leakage. After medication, 5 mL normal saline was instilled and the same procedure described above was adopted for sampling the vaginal secretions at the time interval specified for each woman. Thus, each woman was subjected to the sampling procedure twice, once before and once after medication at one of the specified time intervals of 0.25, 0.5, 0.75 and 1.0 h.

The pH of the vaginal secretions of all the tested women was measured before and after medication at the specified time interval by touching pH indicator paper to the vaginal mucus.

Analysis of metronidazole in vaginal secretions

Concentrations of metronidazole were measured in vaginal secretions using the sensitive and specific high-performance liquid chromatographic (HPLC) technique described by Nilsson-Ehle et al (1981). The vaginal secretion sample was centrifuged at 1500 g and 0.5 mL of the clear supernatant was mixed with an equal volume of 30% trichloroacetic acid. The mixture was vortexed for 10 s and centrifuged at 1800 g for 5 min. An aliquot (200 μL) of the clear supernatant was suitably diluted with the mobile phase and a 20-μL sample was injected onto the HPLC system. The HPLC system consisted of a Waters autosampler (Model 717 plus), solvent delivery system (Model 600), tunable absorbance UV/visible detector (Model 486) and Millennium 2010 chromatography manager. The separation was achieved using a reversed-phase column (Supelcosil ODS, 15×0.46 cm, 5 μm). The column effluent was monitored at 320 nm and the eluent flow rate was 2 mL min^{-1}. The mobile phase consisted of 0.005 M monobasic potassium phosphate (pH 4), methanol and acetonitrile (97:2:1). Calibration curves were constructed in vaginal secretions by spiking the blank samples with the standard amounts of metronidazole. The calibration curves were linear over the range of 1–50 $\mu g\,mL^{-1}$. The sensitivity of the assay under these conditions was 0.5 $\mu g\,mL^{-1}$ in vaginal secretions. The intra-day precision was determined by assaying six samples; the coefficients of variation were 2.0% and 1.0% at concentrations of 1.0 $\mu g\,mL^{-1}$ and 10.0 $\mu g\,mL^{-1}$, respectively. The day-to-day reproducibility of the assay was determined on six separate days; the coefficients of variation were 5.0% and 3.0% at concentrations of 1.0 $\mu g\,mL^{-1}$ and 10.0 $\mu g\,mL^{-1}$, respectively.

Statistical analysis

Analysis of variance was employed in the statistical analysis of the determined parameters in this study using MINITAB statistical software (Minitab release 13.1). Statistical significance was defined at $P < 0.05$.

The rheological data was analysed using one-way analysis of variance combined with Tukey's test to assess

differences between the three gel products. The effects of metronidazole on human sperm were analysed by analysis of variance general linear model. The considered factors were time and drug concentration and the considered responses were percentage motility and percentage viability. Individual differences between means were examined using Tukey's test. The effects of patient group and time (factors) on the concentrations of metronidazole in vaginal secretions (response) were analysed using analysis of variance general linear model followed by Tukey' test. One-tailed Student's t-test was used to assess differences in pH of the vagina before and after medication at all time intervals for both groups.

Results and Discussion

Formulation design

This study aimed to formulate metronidazole in a suitable form for vaginal contraception. The use of gel may be advantageous in presenting the drug in a soluble form and in attaining a relatively high viscosity, enhancing the efficiency of the product to immobilize sperm. Methyl cellulose was chosen as the gel vehicle because of its safe use for vaginal application as well as its compatibility with metronidazole, acetic acid and vaginal secretions. Acetic acid was used in the designed formula for several reasons. (i) To enhance the solubility of the drug in the gel formulation. Metronidazole is slightly soluble in water (1% w/w) and consequently in methyl cellulose gel. Metronidazole is a basic compound and thus more soluble in an acidic environment (Windholz et al 1983). The use of 25 g of glacial acetic acid was sufficient to completely dissolve 5 g of metronidazole. The addition of this solution to 70 g of methyl cellulose gel results in the preparation of a transparent gel product. Thus, a high drug concentration of 5% w/w could be presented in solution in the formulated gel. (ii) To enhance the spermicidal activity of metronidazole gel. The dilute form of glacial acetic acid has been used as a spermicidal as well as antibacterial, antifungal and antiprotozoal in vaginal gels and douches (Reynolds 1993). (iii) The physiological compatibility of acetic acid since it is a natural component of vaginal secretions (Flowers et al 1979). (iv) Its protective effects against sexually transmitted diseases especially HIV (Reynolds 1993).

Rheological characteristics

Figure 1 shows the rheograms of 3% methyl cellulose placebo gel, acid gel (25% glacial acetic acid) and metronidazole acid gel (5% drug and 25% acid). All the tested samples showed pseudoplastic flow. To compare between the pseudoplastic flow of the tested samples, the following exponential formula was applied:

$$F^N = KG \quad (1)$$

$$\text{Log } G = N \log F - \log K \quad (2)$$

F, G, N and K are shearing stress (Pa), shearing rate (s^{-1}), constant and consistency coefficients ($Pa^N.s$), respectively. The calculated rheological parameters for the tested samples are listed in Table 1. All the tested systems fit equation (2) as reflected by the values of R^2. Values of N and K for the placebo gel are decreased significantly ($P < 0.05$) by the addition of acid, rendering the system less viscous and more Newtonian. The addition of drug to the acid gel significantly ($P < 0.05$) increases the values of

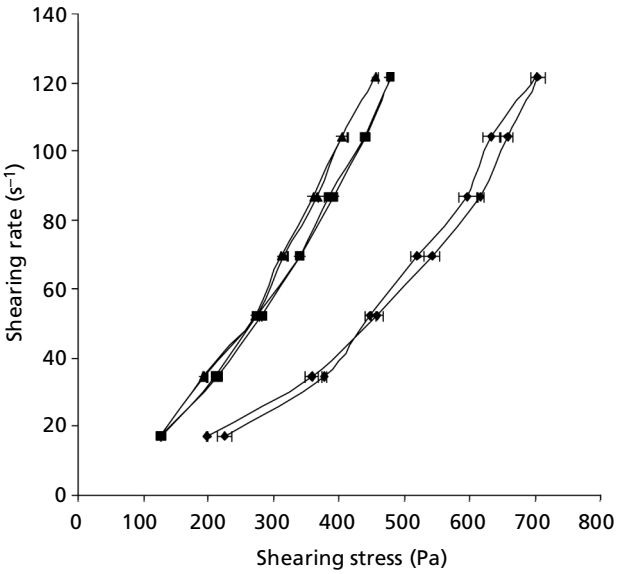

Figure 1 Flow curves for methyl cellulose placebo gel (◆), acid gel (■) and metronidazole acid gel (▲) at 20 °C.

Table 1 Rheological parameters of the tested pseudoplastic systems.

Sample	R^2	N	K ($Pa^N.s$)
Placebo gel	0.9821 ± 0.0027	1.5244 ± 0.0080	201.57 ± 5.47
Acid gel	0.9975 ± 0.0007	1.4617 ± 0.0046[a,b]	70.98 ± 2.66[a,b]
Metronidazole acid gel	0.9965 ± 0.0002	1.5135 ± 0.0034	87.46 ± 3.51[a]

Data are mean ± s.d., n = 3. [a]Significantly different compared with placebo gel ($P < 0.05$); [b]significantly different compared with metronidazole acid gel ($P < 0.05$).

N and K compared with those of the acid gel. Statistical analysis revealed non-significant differences between the N values of placebo gel and metronidazole acid gel. The up and down flow curves reflect thixotropy of the placebo gel, whereas other systems showed no apparent thixotropic characteristics.

The rheology of the vaginal gel could be considered one of the most important factors affecting gel distribution and retention time in the vagina. In addition, consistency of the vaginal product for local contraception has a direct effect on the migration of sperm (Flowers et al 1979). As the consistency of the applied product increases, its efficiency as a contraceptive agent may increase as a result of becoming more tenacious and more resistant to sperm migration and consequently decreasing the capability of sperm to reach the site of fertilization. The consistency of the seminal fluids after liquefaction is 6.54 cP at 20 °C (Diem 1962), which is very low compared with the consistency of the prepared gels. The determined consistency coefficients of the metronidazole acid gel and acid gel were 87.46 and 70.98 Pa^N.s, respectively. These values may be sufficient to enhance the viscosity of the medium through which the spermatozoa migrate.

Spermicidal activity of metronidazole and the gel products

The effect of different concentrations of metronidazole on percentage motility and percentage viability of human sperm in presence of 1 g placebo gel was studied and the results are given in Table 2. According to the standards of the International Planned Parenthood Federation's Agreed Test for Total Spermicidal Power, the product is acceptable if it completely immobilizes all sperm at 40 s and if no sperm are revived after 30 min. A metronidazole concentration of 0.2% w/v showed complete immobilization of sperm within 1 min and no sperm were revived after 10 min (Table 2). Thus, 0.2% w/v could be considered the minimum spermicidal concentration of metronidazole in the presence of placebo gel. This finding agrees with that previously reported by Omar (1995), who performed the same test in the absence of placebo gel and found that the evaluated minimum inhibitory concentration of metronidazole as a spermicide was 0.2% w/v. This result proves that the spermicidal activity of metronidazole is not affected by the presence of placebo gel. A metronidazole concentration of 0.3% w/v is the minimum concentration that showed instant immobilization and death of all the sperm (within 30 s). Statistical analysis revealed significant differences ($P < 0.05$) between the effects of blank and different drug concentrations on percentage motility and percentage viability of sperm. There were significant differences ($P < 0.05$) between the values of percentage motility shown by the 0.2% drug concentration (after 1 min) and those shown by the 0.1% drug concentration at the same time intervals. There were no significant differences between the values of percentage viability shown by the two concentrations. The effect of the 0.3% drug concentration was significantly different ($P < 0.05$) from the effects of the 0.1 and 0.2% drug concentrations on the percentage motility and percentage viability of sperm at 0.5 min. At subsequent time intervals, the 0.3% drug concentration showed significant differences ($P < 0.05$) with 0.1% drug concentration and non-significant differences with 0.2% drug concentration concerning both percentage motility and percentage viability.

The spermicidal activity of the acid gel and the metronidazole acid gel was studied and the results showed instant immobilization and death of all sperm (within 30 s) by both products. Thus, according to the requirements of standards adopted by the International Planned Parenthood Federation, the two products are acceptable as spermicides.

Concentrations of metronidazole in the vaginal discharges

In-vitro tests are a preliminary step to predict the in-vivo performance of the tested products. However, this test can

Table 2 Effect of different concentrations of metronidazole on the percentage motility and percentage viability of human sperm.

Concentration (% w/v)	Effect	Time (min)								
		0.5	1	2	6	10	15	20	25	30
0.0 (blank)	% Motility	85 ± 5.0	81.7 ± 2.9	81.7 ± 2.9	75 ± 5.0	75 ± 5.0	68.3 ± 2.9	66.7 ± 5.8	63.3 ± 2.9	61.7 ± 2.9
	% Viability	98.3 ± 2.9	96.7 ± 2.9	96.7 ± 2.9	96.7 ± 2.9	96.7 ± 2.9	96.7 ± 2.9	96.7 ± 2.9	96.7 ± 2.9	95 ± 5.0
0.1	% Motility[a]	35 ± 18.0	25 ± 8.7	20 ± 10	18.3 ± 7.6	1.7 ± 2.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	% Viability[a]	41.7 ± 12.6	30 ± 17.3	21.7 ± 22.5	18.3 ± 23.6	10 ± 17.3	6.7 ± 11.5	0 ± 0	0 ± 0	0 ± 0
0.2	% Motility[a]	23.3 ± 15.3	0 ± 0[b]	0 ± 0[b]	0 ± 0[b]	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	% Viability[a]	40 ± 13.2	26.7 ± 20.8	10 ± 17.3	6.7 ± 11.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.3	% Motility[a]	0 ± 0[b,c]	0 ± 0[b]	0 ± 0[b]	0 ± 0[b]	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	% Viability[a]	0 ± 0[b,c]	0 ± 0[b]	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Data are mean ± s.d., n = 3. [a]Significantly different compared with blank at all time intervals ($P < 0.05$); [b]significantly different compared with the 0.1 concentration at the same time interval ($P < 0.05$); [c]significantly different compared with the 0.2 concentration at the same time interval ($P < 0.05$).

not simulate factors that influence product dispersion in the vagina, interaction with vaginal secretions, or sperm transport. Clinical studies are therefore necessary to evaluate efficacy in-vivo. The main aim of this study was to determine the effective drug concentration in vaginal secretions after gel application. The reduction in drug concentration due to dilution by the ejaculated semen during normal human coitus should be taken into consideration. To simulate this dilution, 5 mL normal saline was instilled into the vagina before each sample collection. The reported volume of human ejaculate ranges from 0.2 to 6.6 mL, with an average of 3.4 mL (Diem 1962). The vaginal secretions have a considerable effect on the efficacy and concentrations of agents placed in the vagina. There may be a reduction in the effective drug concentration within the vagina due to dilution by vaginal secretions and inactivation of the drug by vaginal bacteria (Ralph & Clark 1978). This study was conducted to ensure that the effective drug concentration in the vagina over 1 h after medication was above the determined minimum spermicidal concentration of the drug (0.2% w/v).

The average drug concentrations in vaginal samples after application of 1 g vaginal gel are given in Table 3. The results were statistically analysed and significant differences ($P < 0.05$) were found between the drug concentrations in the infected and healthy groups at all time intervals. The lower drug concentrations in the infected group may be due to dilution by the excess vaginal discharge and inactivation by the abundant vaginal bacteria in case of vaginitis. The decrease in drug concentration in response to time was not significant except between the drug concentrations at 15 and 60 min ($P < 0.05$) in the two groups. This may indicate good retention of the gel in the vagina. The rheological characteristic of the vaginal gel may be considered the main factor responsible for gel distribution and retention time in the vagina. The significant drop in drug concentration after 60 min may be due to time-dependent drug absorption, accumulation in vaginal tissues (Venkateshwaran & Stewart 1995), leakage from the vagina and/or inactivation by vaginal bacteria. Several studies revealed that vaginal metronidazole is slowly absorbed and its systemic bioavailability is variable according to the applied dose and dosage form, and approximates 20% (Chien et al 1982; Alper et al 1985; Cunningham et al 1994). This reflects a relative barrier effect of the vagina that may allow for an increased local effect of metronidazole within the vagina. The lowest drug concentrations were obtained after 60 min (0.388% and 0.298% in healthy and infected groups, respectively). These concentrations are still higher than the determined minimum spermicidal concentration of the drug (0.2%). However, it is preferable to insert the gel into the vagina shortly before intercourse since its duration of effectiveness may be approximately 1 h.

After vaginal medication, no irritation was reported by the healthy women, but 10 infected women complained of an itching and burning sensation during the first 10 min. Thereafter, there was a gradual relief of these symptoms and complete relief was achieved within 1 h. This sensation may be owing to the highly inflamed vaginal wall as a result of severe vaginal infection.

pH values of the vaginal secretions

The pH values of the vaginal secretions are given in Table 4. For statistical evaluation, one-tailed Student's t-test was applied. There were significant differences between the pH of the vagina before and after medication at all time intervals and for both healthy and infected groups. Before medication, the average pH ranged from 4.3 to 4.8 and from 5.28 to 5.64 in the healthy and infected groups, respectively. After medication, the average pH dropped to a range of 4–4.33 for the healthy group and 4–4.17 for the infected group. This drop returns the pH of the vagina to its normal values (3.5–4.2) and enhances the spermicidal effect within the vagina since sperm survive poorly at low pH (Flowers et al 1979). This low pH may result from the acetic acid (pKa value of 4.74) and metronidazole included in the gel.

Metronidazole may inhibit the production of amines by certain anaerobic vaginal bacteria that are abundant in vaginitis (Chen et al 1979). These amines are responsible

Table 3 Average concentrations of metronidazole in vaginal secretions of healthy and infected women after vaginal application of 1 g of the gel containing 50 mg metronidazole.

Time (min)	Drug concentration ($\mu g\,mL^{-1}$)	
	Healthy group	Infected group
15	5084 ± 1181	3846 ± 883^{a}
30	4543 ± 962	3431 ± 1047^{a}
45	4161 ± 1024	3229 ± 892^{a}
60	3880 ± 691^{b}	$2985 \pm 816^{a,b}$

Data are mean ± s.d., n = 12. [a]Significantly different compared with the healthy group at the same time interval ($P < 0.05$); [b]significantly different compared with the same group at 15 min ($P < 0.05$).

Table 4 pH values of vaginal secretions in healthy and infected groups before and after vaginal medication.

Time (min)	Group	pH		*P* value
		Before	After	
15	Healthy	4.30 ± 0.35	4.00 ± 0.0	< 0.001
	Infected	5.28 ± 0.55	4.00 ± 0.0	< 0.001
30	Healthy	4.86 ± 0.24	4.03 ± 0.11	< 0.001
	Infected	5.36 ± 0.72	4.06 ± 0.20	< 0.001
45	Healthy	4.59 ± 0.47	4.00 ± 0.0	< 0.05
	Infected	5.38 ± 0.75	4.12 ± 0.30	< 0.001
60	Healthy	4.83 ± 0.93	4.33 ± 0.55	< 0.05
	Infected	5.64 ± 0.82	4.17 ± 0.33	< 0.001

Data are mean ± s.d., n = 12.

for the elevated pH in the vagina of the infected group. This may explain the higher drop in average vaginal pH of the infected group compared with that of the healthy group. The drop in pH in the healthy group may be due to the effect of acetic acid, whereas that in the infected group may be due to both effects of the acid and the drug.

Conclusions

The designed vaginal contraceptive was optimized by using a newly developed spermicidal agent, increasing the drug concentration and combining two active agents (metronidazole and acetic acid) in a gel form. The spermicidal activity of metronidazole was investigated in-vitro using human sperm and the determined minimum spermicidal concentration was 0.2% . The results showed that the designed metronidazole acid gel maintained the pH of the vagina at its low normal value and achieved spermicidal drug concentrations in the vagina over 1 h after vaginal application of 1 g of the gel. The product may exert a dual protective activity against sexually transmitted diseases and unplanned pregnancy.

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