Use of mandelic acid condensation polymer (SAMMA), a new antimicrobial contraceptive agent, for vaginal prophylaxis

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Objective: To assess the contraceptive properties, antimicrobial activity, and safety of mandelic acid condensation polymer (SAMMA).

Design: Experimental study of SAMMA’s in vitro and in vivo properties.

Setting: Academic research laboratories.

Patient(s): Healthy volunteers for semen donation in an academic research environment.

Intervention(s): Inhibition of sperm function indicators, conception, sexually transmitted infection–causing pathogens (including HIV), and lactobacilli was evaluated. Safety indicators were studied.

Main Outcome Measure(s): Quantitation of SAMMA’s effect on microbial infectivity or multiplication and on sperm function in vitro; evaluation of contraceptive efficacy in vivo; assessment of safety in vitro and in vivo.

Result(s): Mandelic acid condensation polymer is not cytotoxic toward lactobacilli, microbial host cells, and spermatozoa. The compound inhibits hyaluronidase and acrosin, induces sperm acrosomal loss, and is contraceptive in the rabbit model. Mandelic acid condensation polymer prevents infectivity of HIV and herpesviruses 1 and 2 and, to a lesser extent, of Chlamydia trachomatis. It inhibits the multiplication of Neisseria gonorrhoeae. Mandelic acid condensation polymer is not mutagenic, has low acute oral toxicity, and is safe in the rabbit vaginal irritation assay.

Conclusion(s): Mandelic acid condensation polymer inhibits sperm function, is contraceptive, has broad-spectrum antimicrobial activity, and is highly safe. Further development as a microbicide is warranted.

Key Words: Contraception, spermatozoa, sexually transmitted infection, HIV, toxicology, microbicide

Microbicides are antimicrobial products and formulations. They are intended for topical self-administration before sexual intercourse to protect against the transmission of HIV and other sexually transmitted infection–causing microbes. Microbicides can be used by women without requiring negotiation with their sex partners. This is an important property because condom-dependent protection has been unsuccessful in halting the spread of AIDS and other sexually transmitted infections. Women are particularly vulnerable to the acquisition of AIDS and sexually transmitted infections. In 1998, 43% of AIDS cases or HIV infections were in women. The percentage of infected women is increasing in the approximately 16,000 new cases of HIV infection that occur every day (1). Vaginal HIV transmission is particularly effective when lesions are present in the vagina or cervix, as caused, for instance, by non-HIV sexually transmitted infections (2). In 1995, >333 million new cases of non-HIV sexually transmitted infections occurred worldwide (3). Therefore, the preferred microbicides should have broad-spectrum antimicrobial activity.
The world population continues its steep rise, leading to serious overpopulation problems in certain regions (4). Consumer preference studies suggest that most women worldwide prefer a vaginal prophylactic product to be both antimicrobial and contraceptive (5, 6). For maximal use, a microbicide should also be contraceptive, although noncontraceptive microbicides are also needed in case a woman desires pregnancy.

Originally, marketed vaginal contraceptive products containing the surfactant and detergent nonoxynol-9 were thought to be useful as microbicides because nonoxynol-9 has broad-spectrum antimicrobial activity in vitro. However, clinical trials have not supported this contention (7–9). The most recent study suggests that a formulation with nonoxynol-9 can actually enhance the transmission of HIV (10). Nonoxynol-9 and other surfactants are potent cytotoxins that can destroy vaginal and cervical cells (11) and inactivate the protective vaginal microbes (lactobacilli) (12, 13). Frequent use of nonoxynol-9 products can cause irritation, inflammation, and ulceration of the vagina and cervix (14–16). This, in turn, promotes HIV entry. Lactobacilli maintain the vagina at a pH of 3.5 to 4.5 by producing lactic acid and H2 O2. Human immunodeficiency virus and a number of other sexually transmitted infection–causing microorganisms are inactivated at this acidic pH (17, 18). Inactivation of lactobacilli removes a vaginal barrier against infection.

On the basis of these considerations, the most useful microbicide inhibits HIV and other sexually transmitted infection–causing microbes, is contraceptive but not cytotoxic, and has no effect on vaginal and cervical cells or lactobacilli. With this goal in mind, the Program for the Topical Prevention of Conception and Disease (TOPCAD), a university-based, not-for-profit program centered in Chicago, Illinois, focused its efforts on the discovery and development of noncytotoxic, broad-spectrum, antimicrobial contraceptive agents. The underlying assumption was that tissue and cell entry by sexually transmitted infection–causing microorganisms are inactivated at this acidic pH. A formulation with nonoxynol-9 can actually enhance the transmission of HIV (10).

Mandelic acid condensation polymer is contraceptive in the rabbit model. Furthermore, the compound has no mutagenic activity in vitro and has low acute toxicity in rats. It is safe when applied for 10 consecutive days to the rabbit vagina.

MATERIALS AND METHODS

**Materials**

Mandelic acid condensation polymer was synthesized by TOPCAD using sulfuric acid treatment of mandelic acid. It is odorless and essentially colorless. It is highly soluble in aqueous media but essentially insoluble in organic solvents and is not a surfactant or chelator. A gel formulation containing 4% (40 mg/mL) SAMMA was prepared in a base consisting of carboxymethyl cellulose, propylene glycol, lactic acid, sorbic acid, and methylparaben (preservative) in a proprietary mixture, with a final pH of 4.80 and a viscosity of 34,000 centipoise at 37°C. The pH of the formulation was acidic to match the acidic pH of the vagina.

**Biological Studies**

**Sperm Function Inhibition Tests**

Human semen was collected according to a protocol approved by the institutional review board. Informed consent was obtained from all donors. Inhibition of sperm motility, hyaluronidase, acrosin and cervical mucus penetration, and the stimulation of acrosomal loss were measured as detailed elsewhere (19, 20). Briefly, sperm motility inhibition was tested by a modification of the Sander-Cramer assay (21), observing spermatozoa 30 seconds after a semen sample was mixed with different dilutions of the test compound. Data were reported as percentage of total spermatozoa with tail movement. Hyaluronidase inhibition was assessed by incubating testicular hyaluronidase with the test agent for 10 minutes before colorimetric measurement of hyaluronic acid hydrolysis (22). Data were reported as the change in absorbency at 545 nm caused by product formation.

Acrosin was isolated from human spermatozoa and incubated with the test agent before measuring enzyme hydrolysis of the ethyl ester of N-α-benzoyl-γ-arginine (23). Data were presented as milli-International Units (mIU) of enzyme activity per milligram of protein. Acrosomal loss (disruption of the sperm acrosome) was tested by first mixing washed human spermatozoa with SAMMA. After 15 minutes, the spermatozoa were fixed with buffered glutaraldehyde and stained with Bismarck Brown and Rose Bengal (24). Acrosomal status was observed microscopically. Data were compared with maximal acrosomal loss induced by ionophore A-23187 (25). Values were reported as percentage maximal acrosomal loss.

The effect of SAMMA on the ability of spermatozoa to penetrate cervical mucus was measured by mixing washed,
diluted spermatozoa with SAMMA and placing a Penetrak tube (bovine cervical mucus; Serono Diagnostics, Serono, Italy) into the mixture for 30 minutes (20). The tube was removed and examined microscopically for the length of the tube traversed by the most advanced motile spermatozoa. This distance was compared with that achieved by the most advanced spermatozoa from a sample that was treated in the same fashion but in the absence of SAMMA (control). Distance of migration achieved by the spermatozoa in the test solution was reported as a percentage of the distance achieved by the control spermatozoa.

**Contraception Assays**

All animal studies were approved by the institutional animal care and use committee and performed according to the Guide for Care and Use of Laboratory Animals. Details of the protocol have been presented elsewhere (19, 20). Briefly, semen was collected from New Zealand white rabbits with an artificial vagina, washed by mild centrifugation and resuspension in modified Tyrode’s albumin, lactate, and pyruvate (TALP) medium without albumin, and adjusted with TALP medium to a sperm concentration of approximately $62 \times 10^6$ per milliliter. Initial experiments have shown this concentration to consistently give maximal conception when 0.5 mL (approximately $31 \times 10^6$ spermatozoa) is placed vaginally in the rabbit. Insemination with a larger number of spermatozoa reduces the sensitivity of the assay.

One of two procedures was performed next. When the direct effect of the SAMMA on spermatozoa was determined, the gametes were incubated with 5 mg/mL SAMMA dissolved in TALP medium for 15 minutes. This mixture, containing approximately $31 \times 10^6$ spermatozoa, was inseminated into the vagina of a superovulated rabbit (without formulation in the vagina). When the contraceptive effect of the SAMMA gel was tested, 0.75 mL of the formulation was placed vaginally with a plastic insemination tube past the pelvic bone, followed 15 minutes later by insemination with 0.5 mL (approximately $31 \times 10^6$ washed spermatozoa) of the formulation in the same vaginal location. In both procedures, the rabbits were killed approximately 28–34 hours after insemination, and the oocytes were flushed from the oviducts and examined microscopically for cleavage (embryo formation). Data were reported as the percentage of recovered oocytes that were fertilized for each rabbit.

**Antimicrobial Tests**

The effect of SAMMA on HIV, HSV-1 and HSV-2, gonococci, and chlamydia was tested as described elsewhere (19, 20, 26). The following is a brief description of these assays. The effect of SAMMA on HIV-1 was determined by a viral-binding assay. Human immunodeficiency virus-1 (strain IIIb) was incubated with MT-2 lymphoblastic cells and serial dilutions of SAMMA for 2 hours (19). Virus and compound were removed, and the cells were resuspended in fresh medium and incubated for an additional 6 days at 37°C. Virus-induced cell death was quantified by a dye reduction assay. Compound and cells with no virus were coincubated to measure SAMMA’s cytotoxicity. Data at each concentration of SAMMA were calculated as percentage control viral titer, and the values were reported as averages.

A plaque reduction assay was used to determine the effect of SAMMA on infectivity of HSV-1 (strain 17) and HSV-2 (strain 333) using Ca$K$ski cells (human cervical cell line, ATCC) (26). Virus (200–500 plaque-forming units per well) was mixed with serial dilutions of SAMMA immediately before Ca$K$ski cells were inoculated at 37°C. After a 2-hour adsorption and penetration period, the inoculum was removed, and cells were washed and overlaid with medium containing methylcellulose. Plaques were visualized by immunostaining (blue-plaque assay) after 48 hours. Values were expressed as average viral titer (plaque-forming units per milliliter).

For the gonococcal assays, serial concentrations of SAMMA were mixed with gonococci agar, and the agar was poured onto Petri dishes. The dishes were inoculated with N. gonorrhoeae, strain MS11 (isolated from an uncomplicated case of gonorrhea); then they were incubated overnight, and the colonies were counted (26). Data were reported as average bacterial titer (colony-forming units per milliliter).

Inhibition of chlamydia infectivity was tested by adding C. trachomatis elementary bodies to HeLa cell monolayers in the presence or absence of different concentrations of SAMMA (26). After 1 hour, the monolayers were washed to remove SAMMA and unbound chlamydia. After a 48-hour incubation period, medium was removed, the HeLa cell monolayers were fixed and treated with fluorescein-conjugated antibody, and the inclusions were visualized with a fluorescent microscope. Data were reported as bacterial titer (inclusion-forming units per milliliter).

Lactobacillus inhibition tests were performed as detailed elsewhere (19). Briefly, Lactobacillus gasseri was cultured in Lactobacillus MRS broth under anaerobic conditions in the presence or absence of different concentrations of SAMMA. Culture growth was determined turbidometrically. Data were recorded as absorbencies at 550 nm over time.

Cytotoxic studies with host cells were performed by exposing Caski cells (HSV assay) for 20 hours at 37°C at various concentrations of SAMMA, using the cell titer 96-well kit–cell proliferation assay. The number of viable cells was determined by non-radioactive substrate absorbance at 490 nm.

**Safety and Toxicity Tests**

Detailed information on these tests can be found elsewhere (20). The following is a brief description of the methodology used.
**Bacterial Reverse Mutation Assay (AMES Test)**

The test was performed by BioReliance (Rockville, MD). Mandelic acid condensation polymer was dissolved in water at different concentrations. The mutagenic potential of these SAMMA solutions was evaluated by the plate incorporation method (27). *Salmonella typhimurium* (strains TA98, TA1000, TA1535, and TA1537) and *Escherischia coli* (strain WP2 uvrA) were used in the presence and absence of Aroclor-induced rat liver S9. The maximum dose tested was 5 mg per plate.

**Limit Test for Acute Oral Toxicity**

Acute oral toxicity of SAMMA was determined by the limit test (28). The study was performed at the Biologic Safety Research Laboratory (Maywood, IL) under the direction of one of the investigators (D.W.). Mandelic acid condensation polymer was prepared as a 33% solution in distilled water and was administered by oral gavage as a single dose of 5.0 g per kilogram of body weight to five adult male and five adult female rats (Sprague-Dawley). The animals were observed for pharmacotoxic signs and mortality during a 14-day post-administration observation period. A gross necropsy examination was performed on all animals at the end of the 14-day observation period.

**Rabbit Vaginal Irritation Assay**

Vaginal irritation testing was performed with 4% SAMMA in gel formulation at Biologic Safety Research Laboratory (Maywood, IL) under the direction of one of the investigators (D.W.). A procedure modified from that of Gad and Chengelis (28) was used. Thirty mature female New Zealand White rabbits were divided into three test groups. One group served as untreated control. The other groups were treated vaginally for 10 consecutive days with either SAMMA gel or placebo (1 mL per rabbit per day). All animals were weighed on the 1st day of dosing and every 7th day thereafter. The animals were observed at least twice daily for moribundity or mortality. Observations were also made of vaginal bleeding and discharge, appearance, behavior, and pharmacotoxic signs, approximately 0.5 hour and 4 hours after dosing. Detailed physical observations were made daily. The animals were killed 24 hours after the final vaginal dose of gel was administered, and the vagina was collected, fixed, and sectioned. Three sections from different portions of the vagina were studied histopathologically. The tissue sections were evaluated for the presence and severity of the following: [1] epithelial ulceration; [2] vascular congestion; [3] leukocyte infiltration; and [4] edema. The severity of the changes in each category was scored from 0 to 4, and the scores were totaled. The scores for each vaginal section were added up and divided by three for each rabbit. A mean score of 1–4 represents minimal irritation; 5–8, mild irritation; 9–11, moderate irritation; and 12–16, marked irritation.

**RESULTS**

**Lack of Cytotoxicity**

At the highest concentration tested (5 mg/mL), SAMMA had no effect on the growth of lactobacilli. By contrast, 0.5 mg/mL of the commercial spermicide nonoxynol-9 completely inhibited growth.

Mandelic acid condensation polymer had no cytotoxic effects on Caski cells. All cells were viable after 20 hours of exposure to 1 mg/mL SAMMA (the highest concentration tested). Under the same conditions, nonoxynol-9 was highly cytotoxic at 0.01 mg/mL, with all cells being dead. Mandelic acid condensation polymer also had no effect on MT-2 lymphoblastic cells at the highest concentration tested (0.3 mg/mL).

The compound was not cytotoxic toward spermatozoa. It had no significant effect on the percentage of motile spermatozoa, even at the highest concentration evaluated (25 mg/mL). Motility in the presence of 25 mg/mL SAMMA was 62% (n = 4; 90% confidence limits, 53.8%–70.4%), as compared with control motility of 70% (90% confidence limits, 67.6%–72.4%; P > 1). By comparison, nonoxynol-9 completely immobilizes all spermatozoa at a concentration of 0.1 to 0.2 mg/mL (29).

**Inhibition of Sperm Function Indicators**

Mandelic acid condensation polymer effectively inhibited hyaluronidase and acrosin with IC_{50} values of 5.1 and 3.8 μg/mL, respectively (Figs. 1 and 2). The compound induced the loss of the human sperm acrosome at 0.5 μg/mL as effectively as ionophore A23187, a known stimulant of the acrosome reaction (Fig. 3).

Mandelic acid condensation polymer had little or no effect on the ability of human spermatozoa to penetrate bovine cervical mucus in the Penetrak assay. At 1 mg/mL, the highest concentration tested, penetration in the presence of SAMMA was 87% ± 3.6% (n = 13) of the penetration of untreated, control spermatozoa.

**Contraceptive Effect**

Consistent with its inhibition of hyaluronidase and acrosin and its stimulation of acrosomal loss, SAMMA inhibited the fertilizing capacity of spermatozoa (Fig. 4). Fertilization was reduced by >90% when spermatozoa were preincubated with 5 mg/mL SAMMA before artificial insemination. In gel (see Materials and Methods for formulation composition), 4% SAMMA completely prevented conception when the gel was placed vaginally before artificial insemination.

**Antimicrobial Properties**

Human immunodeficiency virus, HSV-1, and HSV-2 were effectively inhibited by SAMMA. The IC_{50} toward HIV was 6.5 μg/mL, with a 3-log reduction (99.9% inhibition) at 64 μg/mL (Fig. 5). The IC_{50}s toward HSV-1 and HSV-2 were 54 and 5.8 ng/mL, respectively; 3-log reductions occurred at 146 and 26.4 μg/mL, respectively.
Mandelic acid condensation polymer inhibited the growth of gonococci. Because of the sharp increase in inhibition between 10 and 100 μg/mL, no IC₅₀ could be calculated. Three-log (99.9%) inhibition occurred at 100 μg/mL. Some inhibition of chlamydia infectivity was found. The compound had no effect at ≤10 μg/mL but inhibited infectivity significantly (P < .001) at 100 μg/mL and at 1 mg/mL. Inhibition at this latter concentration was 37%.

**Safety and Toxicity**

Mandelic acid condensation polymer was tested in the in vitro mutagenicity assay (bacterial reverse mutation assay). No positive response was obtained at even the highest dose (5 mg per plate). Therefore, SAMMA is not mutagenic in this test.

In the acute oral-toxicity studies, the LD₅₀ proved to be > 5 g/kg. A 40% mortality rate was observed (2/5 males and 2/5 females died) when the rats were treated with a single dose of SAMMA of 5 g per kilogram of body weight. No gross changes were observed in the surviving animals when a necropsy was performed at 14 days after dosing.

In the rabbit vaginal irritation studies, no differences in mean body weight or in clinical observations were found between the treatment groups. Histopathologically, the regional and composite group averages of the vaginas were classified as mild for the SAMMA gel-treated group and as minimal for the untreated control groups. Thus, the SAMMA

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**FIGURE 1**

Hyaluronidase inhibition by SAMMA. The extent to which SAMMA inhibited hyaluronidase was evaluated spectrophotometrically with hyaluronic acid as substrate. Mandelic acid condensation polymer was preincubated with enzyme for 10 minutes at ambient temperature before the reaction with substrate was initiated. Values are presented as the average absorbency at 545 nm at each concentration of SAMMA. Error bars represent the standard error of the mean (n = 3). When not apparent, error bars are within the dimension of the symbol. TableCurve 2D, version 5 (SPSS Statistical Software, Chicago, IL) was used to calculate the concentration of SAMMA required to inhibit hyaluronidase by 50% (IC₅₀; 5.1 μg/mL), as well as by 99.9% (3-log reduction; 8.4 μg/mL).

**FIGURE 2**

Human acrosin inhibition by SAMMA. Human acrosin activity was measured spectrophotometrically with N-α-benzoyl-L-arginine ethyl ester (BAEE) as substrate. Mandelic acid condensation polymer was preincubated with enzyme for 5 minutes before the reaction was initiated by addition of substrate. Values are presented as the average enzyme activity, expressed as milliunits per milligram of protein. Error bars represent standard errors of the mean (n = 3–6 at each concentration). Using TableCurve 2D, version 5 (SPSS Statistical Software), the IC₅₀ was calculated to be 3.8 μg/mL and the concentration required for a 3-log (99.9%) reduction in activity to be 360 μg/mL.
Induction of human sperm acrosomal loss by SAMMA. Washed human sperm suspensions were exposed to 0.5 μg/mL SAMMA. After 15 minutes, the spermatozoa were fixed and stained for acrosomal visualization. Data were subjected to arcsine transformation before further analysis. Values are presented as the average percentage of total spermatozoa that lacked acrosomes. Error bars are upper 90% confidence limits (n = 4 per group). For comparative purposes, acrosomal loss in the presence of SAMMA was compared with that in the presence of a maximally stimulating concentration (under conditions of the experiment) of calcium ionophore, A23187, and with that resulting from no additions other than an equal volume of BWW medium. The test compounds had no effect on the percentage of motile spermatozoa (average motilities: control, 62%; SAMMA treated, 57%; A23187 treated, 58%). Bars with different letter designations differ (P<.01, Newman-Keuls multiple-range test).

Contraceptive effect of SAMMA in the rabbit model. Mandelic acid condensation polymer was either added to washed rabbit spermatozoa (final concentration: 5 mg/mL) before artificial insemination or applied vaginally as part of a formulated gel (total: 30 mg of SAMMA), 15 minutes before artificial insemination. After 25–27 hours, oocytes were harvested and scored for fertilization. Data (percentage fertilization) were subjected to arcsine transformation before further analysis. Values are reported as average fertilization per group. Error bars are 90% confidence limits. Sample sizes are as follows: nine for untreated controls (open bar, left), three for placebo-treated rabbits (open bar, right), seven for rabbits inseminated with pretreated spermatozoa (filled bar, left), and four for rabbits treated vaginally with formulated SAMMA (filled bar, right). Values marked with “A” differ from their respective controls (P<.001, t test).

DISCUSSION

A serious need exists for the development of new methodology that can protect women from the sexual acquisition of AIDS and other sexually transmitted infections. Use of vaccines is an excellent approach but is hampered by the rapid mutation of HIV, which may make it necessary to develop new vaccines continuously. In addition, vaccines are generally only effective against a single microbial species or strain. By contrast, vaginal microbicides can be developed with broad-spectrum inhibitory properties toward HIV and other sexually transmitted infection–causing microbes. These microbicides are preferably also contraceptive.

A significant amount of research is ongoing to identify new microbicides (30, 31). The candidate compounds can be divided into two general classes: [1] cytotoxic agents that act...
by causing cell death and [2] noncytotoxic agents that act by preventing tissue and cell invasion (entry inhibitors). Cytotoxic agents under study are generally hampered by the same problems as nonoxynol-9 because their toxic dose is very similar to their biologically effective dose. This may create safety and toxicity problems when used frequently by women, as has been observed with nonoxynol-9 (14–16).

By contrast, entry inhibitors may have high biological effectiveness while being essentially noncytotoxic. Preclinical studies with several entry inhibitors have been completed, and phase I clinical trials have been initiated (31). The entry inhibitors that have entered clinical trial, including cellulose sulfate, polystyrene sulfonate, a naphthalene sulfonate polymer (Pro 2000), carrageenan, and dextrin sulfate, are all sulfonated or sulfated polymers or polysaccharides. It is likely that these sulfated or sulfonated compounds act by a common mechanism to prevent the entry of pathogenic microbes into either or both tissue or host cells (26) and may have comparable clinical efficacy (with variations depending on the formulation and dose of compound). Therefore, it is important to identify new agents with structures that differ substantially from those of the noncytotoxic compounds that have been brought into clinical trial so far. Mandelic acid condensation polymer is such a compound because it has no sulfur group and its structure differs from that of the above-mentioned sulfated or sulfonated polymers or polysaccharides.

During the course of product identification, TOPCAD synthesized and tested SAMMA. The compound is odorless and essentially colorless and is highly soluble in aqueous solutions. These characteristics are important for the development of a consumer-friendly vaginal formulation. Mandelic acid condensation polymer is produced by the sulfuric acid–assisted modification of mandelic acid. The method of synthesis, developed by TOPCAD, should lend itself to bulk manufacture at a reasonable price. This is important if SAMMA is to be considered for distribution to developing nations in which the AIDS and sexually transmitted infection crisis is the greatest.

Mandelic acid condensation polymer does not appear to act on a nonspecific cytotoxic basis, as indicated by its lack of effect on lactobacilli, on the percentage of motile spermatozoa, and on the host cells used in the in vitro microbial-infection assays. These results, as well as the data discussed below, suggest that the compound acts as an entry inhibitor.

The compound effectively inhibits conception in the rabbit model when mixed directly with spermatozoa before vaginal insemination or when placed in gel formulation vaginally before artificial insemination. Mandelic acid condensation polymer is not spermicidal and has little or no effect on sperm entry into cervical mucus. The latter result suggests that forward progressive motility of the spermatozoa is not altered and that the properties of cervical mucus are not changed by SAMMA. The compound most likely prevents the fertilizing capacity of spermatozoa by another mechanism than affecting motility.

The contraceptive effect of SAMMA may be caused by inhibition of acrosomal enzymes involved in the functional activity of spermatozoa. The compound strongly inhibits hyaluronidase and acrosin. Inhibitors of these enzymes are contraceptive in the rabbit model (32, 33). Another reason for the contraceptive effect may be its ability to cause the dispersion of the sperm acrosome, an important organelle for

**FIGURE 5**

HIV-1 inhibition by SAMMA. HIV-1IIIb was coincubated with different concentrations of SAMMA and MT-2 cells for 2 hours at 37°C. After this time, unbound virus and SAMMA were removed by washing the MT-2 monolayer with fresh medium. The MT-2 cells were incubated for an additional 6 days. Virus-induced cell death was quantified with an XTT dye reduction assay, as a decrease in absorbency recorded for the cell control. Each concentration of SAMMA was tested in triplicate. Data at each concentration of SAMMA were calculated as percentage of control viral infection (no inhibitor added) and subjected to arcsin transformation before further analysis. Values are reported as averages, with upper 90% confidence limits. When not apparent, error bars are within the dimension of the symbol. TableCurve 2D, version 5 (SPSS Statistical Software) was used to calculate the concentration of SAMMA required to inhibit viral titer by 50% (IC50; 6.5 μg/mL) and by 99.9% (3-log reduction; 64 μg/mL).


![Graph showing HIV-1 inhibition by SAMMA](image-url)

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successful sperm–oocyte interaction (34). Premature disruption of the acrosome in the vagina, and the release of its oocyte-binding receptors as well as its lytic enzymes, may prevent spermatozoa from fertilizing.

Mandelic acid condensation polymer is also a broad-spectrum inhibitor of a variety of sexually transmitted infection–causing microbes, being highly effective at low concentrations. The compound inhibits the enveloped viruses, HIV, HSV-1, and HSV-2, with IC_{50} ranging from 0.006 to 6.5 μg/mL. Furthermore, the compound inhibits the nonenveloped bovine papilloma virus (BPV), with an IC_{50} of approximately 12–35 μg/mL (Hermonat PL, Anderson RA, Zaneveld LJD, unpublished data). Inhibition of BPV is of particular interest because surfactants such as nonoxynol-9 have no effect on this virus (35). Furthermore, the compound inhibits the growth of N. gonorrhoeae by 99.9% at 100 μg/mL and has some effect on C. trachomatis at 0.1 and 1 mg/mL. Mandelic acid condensation polymer may be acting against one or more of the invasive properties that sexually transmitted infection–causing microbes have in common. However, it does not appear to interfere with important noninvasive microbes because lactobacilli are not inhibited by SAMMA.

Presently, it is not clearly understood by which mechanisms SAMMA is inhibitory toward both sexually transmitted infection–causing microbes and spermatozoa. Similar to the case with spermatozoa, a number of sexually transmitted infection–causing microbes, including HIV, were reported to use hyaluronidase, a proteinase, or both for tissue and cell invasion (36–40). For instance, the target cells of HIV possess a serine proteinase, similar to acrosin and trypsin. This enzyme cleaves glycoprotein 160 to glycoprotein 120, allowing binding of HIV to its host cell (39). Typical inhibitors of acrosin, such as 4′-acetamidophenyl 4-guanidinobenzoate, that prevent conception (33) also prevent the activity of this serine proteinase and the entry of HIV into its host cells (40). Enzyme inhibition may be a common mechanism whereby SAMMA inhibits the functional activity of both spermatozoa and STI-causing microbes.

Glycosaminoglycans, such as heparin sulfate, are important for host cell binding of many sexually transmitted infection–causing pathogens, including herpes simplex virus (HSV), gonococci, chlamydia, and HIV (26, 41). Glycosaminoglycans also appear to have an important role in the sperm acrosome reaction and in the interaction between spermatozoa and the oocyte (42–45). Because SAMMA causes acrosomal loss, it is possible that another common mechanism is SAMMA’s interaction with cell surface glycosaminoglycans.

Safety is an important consideration before a compound is entered into clinical trial. Consistent with its noncytotoxic nature, SAMMA has been safe in the toxicity tests performed so far. It is not mutagenic in the Ames test and has an LD_{50} in the acute oral–toxicity assay of >5 g/kg. Mandelic acid condensation polymer was also safe when administered for 10 consecutive days to the rabbit vagina. These observations are consistent with the clinical use of mandelic acid and some of its derivatives (46, 47). For instance, mandelic acid (administered orally and by direct bladder instillation) and methenamine mandelate (administered orally) are used as urinary antiseptics. Permitted oral doses of the latter in adults are as high as 12 g/d.

This is the first report on an exciting new, noncytotoxic vaginal microbicide candidate whose structure differs from that of other noncytotoxic agents under development. The present observations indicate that SAMMA possesses broad-spectrum antimicrobial activity and is an effective contraceptive. It can be classified as an entry inhibitor. The tests performed so far suggest a high degree of safety. A U.S. patent on the antimicrobial and contraceptive properties of SAMMA has been granted (48). These results support completion of the preclinical studies and evaluation of SAMMA in clinical trials.

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