Development and Evaluation of Microbicidal Hydrogels Containing Monoglyceride as the Active Ingredient

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Abstract □ A number of medium-chain saturated and long-chain unsaturated fatty acids and their monoglycerides were tested against herpes simplex virus (HSV-1) to determine which lipids were most active during a short incubation time. The aim was to find which lipid would be preferable as the active ingredient in a virucidal hydrogel formulation for the purpose of preventing transmission of pathogens to mucosal membranes, particularly sexually transmitted viruses, such as herpes simplex virus and human immunodeficiency virus (HIV), and bacteria, such as *Chlamydia trachomatis* and *Neisseria gonorrheae*. The main strategy was that the formulations would be fast-acting, killing large numbers of virus or bacteria on contact in a short time, preferably causing at least a 10000-fold reduction in virus/bacteria titer in 1–5 min. Monocaprin, the 1-monoglyceride of capric acid, and lauric acid were found to be most active of all the lipids tested, causing a greater than 100000-fold reduction in virus titer in 1 min at a concentration of 20 mM. When tested at a concentration of 10 mM for 1 min, monocaprin was still fully active whereas lauric acid had no or neglible activity. It was concluded that monocaprin was most suitable as the active ingredient in a fast-acting virucidal gel formulation, and several hydrogel formulations containing monocaprin were tested. Formulations where the monoglyceride was dissolved in glycofurol were found to be active against HSV-1. The hydrogel formulations containing 20 mM monocaprin were highly virucidal in vitro and caused a greater than 100000-fold (HSV-1) inactivation of virus in human semen in 1 min. Formulations in dilution 1:10 were cytotoxic in monolayers of CV-1 cells, but they were 10–100 fold less cytotoxic than a commercial product which contains 2% nonoxynol-9.

Introduction

There are several published reports on antiviral and antibacterial activities of lipids.[1−5] Research has shown that enveloped viruses, such as herpes simplex virus type 1 (HSV-1), vesicular stomatitis virus (VSV), and visna virus are inactivated by long-chain unsaturated and medium-chain saturated fatty acids, whereas long-chain saturated and short-chain fatty acids have no or only a minor virucidal effect at the highest concentrations tested. Electron microscopy studies of VSV suggest that fatty acids disrupt the lipid envelope of the virus, but the mechanism is not known.[4]

It has been proposed that fatty acids and monoglycerides may be used as intravaginal microbicides for protection against sexually transmitted diseases.[6] To prevent infection by a sexually transmitted pathogen it is important that the micobicide is fast acting and kills the infectious agent before it has time to infect cells of the genital mucosa. It is also important that the microbicide be solubilized in a pharmaceutical formulation which can be easily and effectively applied to mucosa or skin areas which can serve as entry sites for infectious agents. The objective of the work was the development of hydrogels which could be used as vehicles for microbicidal lipids for the purpose of preventing transmission of pathogens to mucosal membranes, particularly sexually transmitted viruses, such as HSV and HIV, and bacteria, such as *Chlamydia trachomatis* and *Neisseria gonorrheae*. The main strategy was to design fast-acting formulations which kill large numbers of virus or bacteria on contact in a short time, preferably causing at least a 10000-fold reduction in virus/bacteria titer in 1−5 min. The effectiveness of the hydrogel formulations was tested against HSV-1 as a model.

Materials and Methods

Materials—Fatty acids and monoglycerides as well as sodium carboxymethylcellulose (NaCMC) and poly(vinylpyrrolidone) (PVP) were purchased from Sigma Chemical Co., St. Louis, MO. Carbomer (Carbopol 934) was obtained from Nomeco, Copenhagen, Denmark, and hydroxypropylmethylcellulose (HPMC) was from Aldrich Chemical Co. Inc., Milwaukee, WI. All other chemicals were of reagent grade.

Hydrogel Formulations—Hydrogels containing either 10 or 20 mM monocaprin were formulated using as gel-forming agents either NaCMC and PVP (series 1A−M) or carbomer and HPMC (series 2A−D). The concentration of gel-forming agents was such that the viscosity of the gels was comparable. One of the following compounds was used to bring the monoglyceride into solution: glycofurol 75, Tween 80, Chremophor PH40, Chremophor EL, 2-hydroxypropyl-β-cyclodextrin.

Formulations based on 2% w/v NaCMC and 1% w/v PVP were produced by adding 1.0 g of NaCMC and 0.5 g of PVP to a solution of monocaprin. The pH of gel formulation 1A is 7 but for formulations 1B−M the pH is adjusted to 5.0 by the dropwise addition of a lactic acid solution. Finally, purified water is added, bringing the solution to a final weight of 50 g. Immediately after the addition of water, the solution is stirred continuously until a hydrogel is produced. The hydrogel is centrifuged at high speed (>8000 rpm) for 60 min in order to remove air bubbles.

Hydrogels based on 0.5% w/v carbomer and 1% w/v HPMC are produced by dispersing 0.5 g of HPMC in 10 mL of hot (80−90 °C) purified water in a glass beaker. The solution is allowed to cool to about 30−35 °C under continuous stirring at room temperature and then chilled in a refrigerator at about 4 °C for at least 1 h. A 0.25 g amount of carbomer is suspended in 5 mL of purified water at room temperature under vigorous stirring to prevent lumping. The carbomer solution is mixed with the HPMC solution, and then a solution of monocaprin is added. Gelling of the carbomer polymer is induced by raising the pH to approximately 5.5 with the dropwise addition of a 2 w/v % sodium hydroxide solution. The hydrogel is brought to its final weight (50 g) with purified water. Finally, the hydrogel is centrifuged at high speed (>8000 rpm) for 60 min to remove air bubbles.

Cell Cultures and Media—CV-1 cells (African green monkey kidney cell line) were grown in Dulbecco's Modified Eagle Medium

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(D-MEM) with 2 mM L-glutamine, 20 μg/mL of gentamicin, and 10% heat-inactivated fetal bovine serum (FBS). The maintenance medium (MM) for CV-1 cell monolayers was D-MEM with 2% FBS. All media were obtained from GIBCO, Paisley, Scotland.

Virus and Virus Titration—HSV-1 strain MacIntyre was obtained from the American Type Culture Collection, Rockville, MD, and grown in CV-1 cells. Virus stocks with an infectivity titer of $10^{6.5}$–$10^{7.5}$ $CCID_{50}$ (50% cell culture infective dose) per 100 μL were used in the experiments. Virus was titrated by inoculation of 10-fold dilutions in MM into monolayers of CV-1 cells in 96-well microtiter tissue culture plates (Nunc, Roskilde, Denmark). One hundred microliters of each virus dilution were inoculated into quadruplicate wells. The plates were incubated at 37° C in a humidified incubator with 5% CO_2 in air and examined for cytopathic effect daily for 5 days. All virus titers were calculated by the method of Reed and Muench.[7]

Assay of Virucidal Activity of Fatty Acids and 1-Monoglycerides in Medium—The stock lipid solutions were diluted to the desired concentration in MM by vortexing at the highest speed for 10 min. The lipids were thoroughly mixed with equal volumes (200 μL) of HSV-1 in 12 × 75 mm polystyrene round-bottom tubes (Falcon) and incubated for 1 min at room temperature. The action of the lipid was then stopped by immediately diluting the mixture 100-fold in medium. Virus mixed with MM alone and with 2% ethanol in MM served as controls. The virus titers of the mixtures were determined by inoculation of 10-fold dilutions into cell cultures as previously described. The lowest inoculated dilution was 10^{-2} because of toxic effect of the lipids on cells. In such lipid–virus mixtures where no cytopathic effect was detectable in the 10^{-2} dilution the titer was assumed to be $\leq 10^{1.5}$ $CCID_{50}$ per 100 μL. The titer (log_{10}) of a lipid–virus mixture was subtracted from the titer (log_{10}) of the control mixture and the difference, i.e., the reduction in viral infectivity, was used as a measure of the virucidal activity of the lipid.

Assay of Virucidal Activity of Hydrogel Formulations—A hydrogel (200 μL) was placed in a 35 mm tissue culture dish (Nunc), and equal volume of HSV-1 in medium was added. The hydrogel was thoroughly mixed with the virus at room temperature for 1, 5, or 10 min. The mixtures were then immediately diluted 100-fold in medium and titrated in 10-fold dilutions. Virus mixed with medium instead of hydrogel was used as a control. The activity of the hydrogels was calculated as in the experiments with lipid solutions. Virucidal activity against HSV-1 in semen was tested in the same way except that the virus was first concentrated 10-fold by centrifugation in a Sorvall ultracentrifuge at 100000*g* for 90 min and then diluted 1:10 in fresh (<2 h) liquefied human semen. Virus-spiked semen mixed with medium was used as a control.

Evaluation of Toxic Effects of Gel Preparations in Monolayers of CV-1 Cells—Tenfold dilutions of hydrogel formulas were added to monolayers of CV-1 cells, 4 wells per dilution, and the cell layers were examined for lysis or other toxic effects after 1 and 24 h.

HPLC Assay of Monocaprin—The monoglyceride content was determined using a high-performance liquid chromatography (HPLC) component system consisting of a Thermo Separations Products Spectra Series P200 HPLC solvent delivery system, a μBondapak C18 125A 10μm (3.9 × 300 mm) column, a Waters Intelligent Sample Processor (WISP) Model 710B, a Thermo Separations Products SP4400 Integrator, and a Thermo Separations Products Spectra Series UV150 detector. The wavelength was 218 nm, and the mobile phase consisted of acetonitrile, water, and tetrahydrofuran (57:42:1) with the retention time being 2.1 min at 1.25 mL/min flow rate.

Drug Release from Hydrogels—Release of monoglyceride was investigated using a membraneless diffusion cell at 37 °C. Phosphate buffer (pH = 4.5) containing 0.1% 2-hydroxypropyl-β-cyclodextrin was used as the receiver phase. Samples were taken from the receiver phase at regular intervals and filtered through a 0.22 μm membrane filter. After each sampling, the volume was replaced. The amount of monoglyceride released was determined by HPLC using a calibration curve of the monoglyceride in the receiver phase. Each experiment was carried out in triplicate.

Results

Anti-HSV-1 Activity of Fatty Acids and 1-Monoglycerides in MM—In a previous study[4] medium-chain saturated and long-chain unsaturated fatty acids were found to inactivate HSV-1 in 30 min at 37 °C, and 1-monoglycerides were found to be more active than the corresponding fatty acids. In the present study caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), palmitoleic acid (16:1), and oleic acid (18:1) and their 1-monoglycerides were tested against HSV-1 for 1 min at room temperature. In addition, undecylenic acid (11:0) was tested. The purpose of this experiment was to determine which of these compounds would be preferable as the active ingredients in a fast-acting virucidal gel formulation, i.e., would inactivate an enveloped virus such as HSV-1 within 1 min. In the first experiment all of the lipids were tested at a concentration of 20 mM. The results are shown in Figure 1. Of the fatty acids only lauric acid (12:0) and palmitoleic acid (16:1) caused a 100000-fold reduction of virus titer in 1 min. All the other fatty acids had no or a very small effect. Of the monoglycerides, monocaprin (10:0) was the most active, but monolaurin also had considerable activity and caused a 100000-fold reduction in virus titer. The most active compounds were tested further in concentrations of 2.5 mM, 5 mM and 10 mM. The results are shown in Tables 1 and 2. Both lauric and palmitoleic acid lost most of their activity at 10 mM or lower concentrations, whereas the activity of monolaurin was unchanged. Monocaprin had by far the highest activity and caused a greater than 700000-fold reduction of virus titer in 1 min at a concentration of 5 mM (Table 2). The virucidal activities of less active compounds were additive if two or more were mixed together (results not shown).

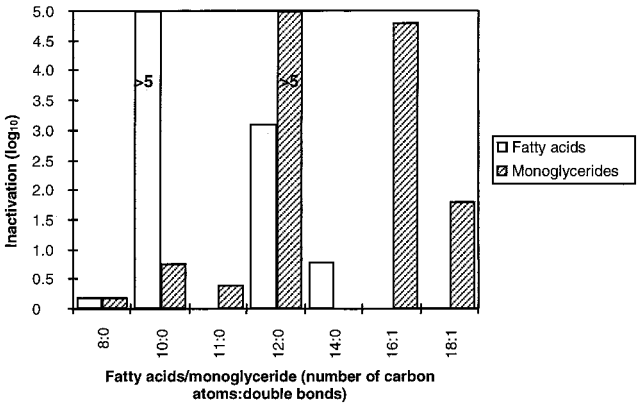

Figure 1—Inactivation of HSV-1 by incubation with an equal volume of 20 mM fatty acid or monoglyceride at room temperature for 1 min. Fatty acids and monoglycerides are indicated by the number of carbon atoms and double bonds. Inactivation is expressed as log reduction of virus titer. The data are the means of at least three experiments.

Table 1—Inactivation of HSV-1 by Incubation with an Equal Volume of Fatty Acid at Room Temperature for 1 min

fatty acid	concn, mM	reduction of virus titer (log_{10})[a]
lauric acid (12:0)[b]	20	≥5.8
	10	0
palmitoleic acid (16:1)	20	4.9
	10	1.6
	5	0
oleic acid (18:1)	20	1.7
	10	0

[a] The data are the means of three experiments. [b] Numbers of carbon atoms: double bonds.

Anti-HSV-1 Activity of Different Hydrogel Formulations Containing Monocaprin—Since monocaprin had the highest virucidal activity against HSV-1 as shown in Tables 1 and 2, this monoglyceride was selected as the active ingredients in hydrogel formulations. The gel-forming agents used were carbomer (series 2A–D) and

Table 2—Inactivation of HSV-1 by Incubation with an Equal Volume of 1-Monoglycerides at Room Temperature for 1 min

monoglyceride	concn, mM	reduction of virus titer (log_{10})[a]
monocaprin (10:0)[b]	20	≥5.8
	10	≥5.5
	5	≥5.5
	2.5	2.2
monolaurin (12:0)	20	3.0
	10	3.4
	5	3.0

[a] The data are the means of three experiments. [b] Numbers of carbon atoms: double bonds.

Table 3—Inactivation of HSV-1 by Incubation at Room Temperature for 1 min with Various Hydrogel Formulations. Series 1A–M Formulations Containing NaCMC and PVP and Series 2A–D Formulations Containing Carbomer and HPMC

gel	method of dissolving monocaprin	monocaprin concn, mM	reduction of virus titer (log_{10})[a]
1A	Glycofurol 75 (pH adjusted to 7)	0	0
		10	2.9
		20	≥5.5
1B	Glycofurol 75 (pH adjusted to 5)	0	0
		0	2.8
		20	≥5.5
1D	Glycofurol 75 and PEG200	0	0.2
		10	2.0
1H	Tween 80	0	0.2
		20	<1.0
1I	Chremophor PH40	0	0.1
		20	<0.8
1L	Chremophor EL	0	0.3
		10	<0.8
1M	2-hydroxypropyl-β-cyclodextrin	0	0
		10	<0.8
2A	Glycofurol 75	0	0
		10	3.2
		20	≥5.5
2B	Propylene glycol	0	0.2
		10	2.2
		20	≥4.2
2D	2-hydroxypropyl-β-cyclodextrin	0	0
		10	<0.8

[a] The data are the means of three experiments.

NaCMC (series 1A−M), both compounds that have good bioadhesive properties and have been used for the formulation of vaginal dosage forms.[8,9] As monocaprin is not soluble in water the hydrogels were formulated in such a way that the monoglyceride was solubilized in the dosage form. It was attempted to use a surface active agent (Tween 80, Cremophor RH40, Cremophor EL) to solubilize the compound, and complex formation with a cyclodextrin (2-hydroxypropy-β-cyclodextrin) was also attempted. Solvents were used to dissolve the monoglyceride (propylene glycol, glycofurol 75) as well as a combination of solvents and surfactants. Table 3 shows the inactivation of HSV-1 by incubation at room temperature for 1 min with hydrogels, with or without monocaprin. The results indicate that both propylene glycol and glycofurol 75 are suitable solvents for monocaprin in hydrogels with either Carbopol or NaCMC as carrier. Addition of surfactants or complex-forming compounds such as 2-HPβCD inhibits the activity of monocaprin in the hydrogels. As propylene glycol can cause irritation of mucosal membranes it was decided to continue the work using hydrogels formulated with glycofurol 75.

Hydrogel formulations 1A, 1B, and 2A were further tested for activity against HSV-1. The hydrogels were mixed with equal volumes of HSV-1, incubated at room temperature for 1 or 10 min, and then titrated as described in Materials and Methods. The results are shown in Table 4. Control hydrogels without monocaprin had an insignificant effect on the virus except hydrogel 2A after incubation for 10 min. In all the hydrogels, monocaprin at 5 mM or higher concentration inactivated the virus more than 100000-fold (>5 log) in 1 min. Monocaprin concentration of 2.5 mM was less active.

Table 4—Inactivation of HSV-1 Mixed with an Equal Volume of Gels 1A, 1B, or 2A without Active Ingredient or with Various Concentrations of Monocaprin and Incubated at Room Temperature for 1 and 10 min

gel	monocaprin concn, mM[a]	incubation, min	reduction of virus titer (log_{10})[b]
1A	0	1	0.4
	0	10	0.6
	2.5	1	<0.8
	2.5	10	2.3
	5	1	≥5.2
	10	1	≥5.2
	20	1	≥5.2
1B	0	1	0.2
	0	10	0.3
	2.5	1	1.0
	2.5	10	2.0
	5	1	≥5.2
	10	1	≥5.2
	20	1	≥5.2
2A	0	1	0.2
	0	10	2.3
	2.5	1	3.3
	2.5	10	≥4.8
	5	1	≥5.2
	10	1	≥5.2
	20	1	≥5.2

[a] The concentration is reduced by half in the final mixture. [b] The data are the means of three experiments.

Table 5—Inactivation of HSV-1 Diluted 1:10 in Semen and Mixed with an Equal Volume of Gels 1A, 1B, and 2A. Incubation Was at Room Temperature for 1, 5, or 10 min[a]

gel	monocaprin concn, mM	incubation, min	reduction of virus titer (log_{10})
1A	0	10	0
	5	10	2.3
	10	1	2.9
	10	5	4.2
	10	10	≥5.0
	20	1	≥5.5
1B	0	10	0
	5	10	1.0
	10	1	2.8
	10	5	4.4
	10	10	≥5.0
	20	1	≥5.5
2A	0	10	0
	5	10	1.6
	10	1	3.2
	10	5	≥5.0
	10	10	≥5.5
	20	1	≥5.5

[a] The data are the means of three experiments.

The virucidal activity of the hydrogels was further tested against HSV-1 suspended in semen rather than in maintenance medium. The results are shown in Table 5. The virucidal activity was considerably less in semen with only the 20 mM concentration causing greater than 100000-fold inactivation in 1 min.

In Vitro Study of Drug Release—The drug release profiles from the different hydrogels are shown in Figure 2. Drug release profile from the 1B hydrogels, containing

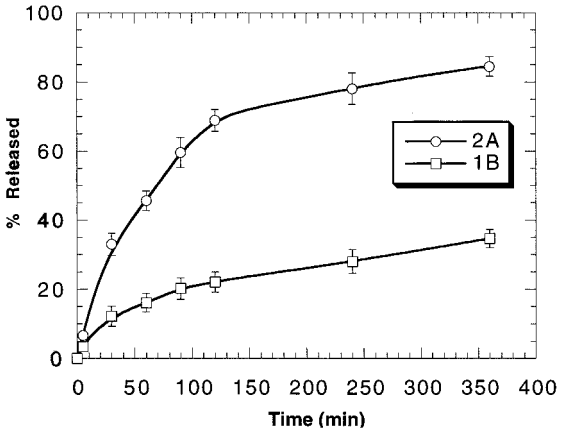

Figure 2—Release of monocaprin from hydrogel formulations 1B (containing NaCMC) and 2A (containing carbomer and HPMC).

Table 6—Cytotoxic Effects of Hydrogel Formulations 1A and 2A,with or without Monocaprin, in Monolayers of CV-1 Cells

		cytotoxic dilution	
gel	monocaprin concn, mM	1 h	24 h
1A	0	$(10^{-1})^a$	10^{-1}
	10	10^{-1b}	10^{-1}
	20	10^{-1}	10^{-1}
2A	0	(10^{-1})	10^{-1}
	10	10^{-1}	10^{-1}
	20	10^{-1}	10^{-1}
Gynol-plus[c]		10^{-2}	10^{-3}

[a] In parentheses: round cells, not lysed. [b] Without parentheses: complete lysis of cells. [c] A commercial (Cilag AG, Schaffhausen,Switzerland) spermicidal gel containing 2% nonoxynol-9 in Povidone K30 and propylene glycol.

NaCMC and PVP as the gelling agents, show a burst effect phenomenon during the first 30 min. Comparison of the release from the two hydrogel bases reveals that the monoglyceride release rate is appreciably higher from the hydrogel 2A containing carbomer and HPMC. This could be due to a less dense gel structure or a different release mechanism in the two hydrogel structures. These results indicate that the release of monocaprin is suitable for prolonged spermicidal and virucidal activity.

Cytotoxic Effect of Hydrogel Formulations in Monolayers of CV-1 cells—Hydrogels containing 10 or 20 mM of monocaprin were found to be cytotoxic in dilution 10^{-1} in virus titrations. Hydrogels 1A and 2A without monocaprin and with 10 or 20 mM monocaprin were inoculated in dilution 10^{-1} onto cell monolayers. The cells were then examined microscopically for cytotoxic effect after 1 and 24 h. Gynol-plus, a commercial contraceptive containing nonoxynol-9, was tested in the same way for comparison. The results are shown in Table 6. Both hydrogel formulations without monocaprin showed some toxic effect after 1 h and complete lysis after 24 h. Hydrogels with monocaprin were more cytotoxic and lysed all the cells in 1 h. No visible toxic effect was seen in 10^{-2} or higher dilutions even after incubation for 4 days (results not shown). A vaginal gel (Gynol-plus) which contains 2% nonoxynol-9, caused a complete cell lysis in 1 h in dilution 10^{-2} and in 24 h in dilution 10^{-3}. It was therefore 10- to 100-fold more toxic to the cell layers than formulations 1A and 2A containing 20 mM (0.5%) monocaprin.

Discussion

In this paper we report novel pharmaceutical formulations in the form of hydrogels of various compositions, which contain a simple lipid, i.e., monocaprin, as the microbicidal ingredient. Monocaprin was selected after a comparison of the virucidal activity of several fatty acids and monoglycerides. In addition to monocaprin, some of these lipids showed considerable activity when suspended in maintenance medium, particularly lauric and palmitoleic acid (Figure 1). However their activities in hydrogel formulations have not yet been studied.

Hydrogels containing monocaprin in concentrations of 5−20 mM are highly active against HSV-1, which was used as a test virus, and cause greater than 100000-fold inactivation of the virus in 1 min. Since some viruses are transmitted by semen, we tested the activity of the hydrogels against HSV-1 suspended in semen and found that a monocaprin concentration of 20 mM was needed for maximum efficacy of the gels. A lower activity of monocaprin in a biological fluid like semen could be expected because previous studies have shown that as much as 10-fold higher lipid concentrations are required for inactivation of virus in human blood than in culture medium.[6] Several enveloped viruses, such as HSV-1, VSV, measles virus, respiratory syncytal virus, cytomegalovirus, visna virus, and HIV-1 have been found to be equally susceptible to lipids.[1,10−12] It was therefore considered appropriate to use HSV-1 as a model in the present experiments to study the rapid microbicidal effect of various lipids. Recent studies on the effect of fatty acids and monoglycerides on *Chlamydia trachomatis*[13] have shown that monocaprin is also the most active in killing this bacterium in a short time and that hydrogel formulations 1A, 1B, and 2A containing 10 mM monocaprin kill *Chlamydis trachomatis* and *Neisseria gonorrheae* at high titers in 5 and 1 min, respectively.[14]

The hydrogel formulations in 10^{-1} dilution were found to be toxic to monolayers of CV-1 cells, but they were 10- to 100-fold less cytotoxic than a commercial spermicidal product containing 2% nonoxynol-9. However, such a comparison must also be carried out in vivo. A preliminary experiment with hydrogel fomulation 1A in mice showed no adverse effect on their vaginal mucosa.[14] Testing of the hydrogel formulations 1A and 2A by the standard rabbit vaginal irritation test showed no irritation of the vaginal mucosa after daily application for 10 days.[14] A comparison of the toxicity of the hydrogels and a vaginal contraceptive gel which contains 4% nonoxynol-9 is now in preparation.

Conclusions

The hydrogel formulations containing 20 mM monocaprin are highly virucidal in vitro and cause a greater than 100000-fold inactivation of virus (HSV-1) in 1 min, even in the presence of semen which considerably reduces the activity at a concentration of 10 mM. Formulations in dilution 1:10 are cytotoxic in monolayers of CV-1 cells, but they are 10- to 100-fold less cytotoxic than a commercial product which contains 2% nonoxynol-9.

References and Notes

1. Isaacs, C. E.; Litov, R. E.; Thormar, H. Antimicrobial activity of lipids added to human milk, infant formula, and bovine milk. *Nutr. Biochem.* **1995**, *6*, 362−366.
2. Kabara, J. J. Fatty acids and derivatives as antimicrobial agents. In: *The pharmacological effect of lipids*.; Kabara, J. J., Ed.; The American Oil Chemists Society: St. Louis, 1978; pp 1−13.
3. Shibasaki, I.; Kato, N. Combined effects on antibacterial activity of fatty acids and their esters against gram-negative bacteria, In *The Pharmacological Effect of Lipids*; Kabara, J. J., Ed.; The American Oil Chemists Society; St. Louis, 1978; pp 15−24.
4. Thormar, H.; Isaacs, C. E.; Brown, H. R.; Barshatzky, M. R.; Pessolano, T. Inactivation of enveloped viruses and killing

of cells by fatty acids and monoglycerides. *Antimicrob. Agents Chemother.* **1987**, *31*, 27−31.
5. Welsh, J. K.; Arsenakis, M.; Coelen, R. J.; May, J. T. Effect of antiviral lipids, heat and freezing on the activity of viruses in human milk. *J. Infect. Dis.* **1979**, *140*, 322−328.
6. Isaacs, C. E.; Kim, K. S.; Thormar, H. Inactivation of enveloped viruses in human bodily fluids by purified lipids, *Ann. N.Y. Acad. Sci.* **1994**, *724*, 457−464.
7. Reed, L. J.; Muench, M. A simple method of estimating 50% end points. *Am. J. Hyg.* **1938**, *27*, 493−497.
8. Knuth, K.; Amiji, M.; Robinson, J. R. Hydrogel delivery systems for vaginal and oral applications. *Adv. Drug Delivery Rev.* **1993**, *11*, 137−167.
9. Park, H.; Robinson, J. R., Mechanism of mucoadhesion af poly(acrylic acid) hydrogels. *Pharm. Res.* **1987**, *4*, 457−465.
10. Isaacs, C. E.; Thormar, H. The role of milk-derived antimicrobial lipids as antiviral and antibacterial agents. In *Immunology of Milk and the Neonate*; Mestecky, J., Plenum Press: New York, 1991; pp 159−165.
11. Thormar, H.; Isaacs, C. E.; Kim, K. S.; Brown, H. R. Inactivation of visna virus and other enveloped viruses by free fatty acids and monoglycerides. *Ann. N.Y. Acad. Sci.* **1994**, *724*, 465−471.
12. Isaacs, C. E.; Thormar, H.; Pessolano, T. Membrane disruptive effect of human milk: Inactivation of enveloped viruses. *J. Infect. Dis.* **1986**, *154*, 966−971.
13. Bergsson, G.; Arnfinnsson, J.; Karlsson, S. M.; Steingrímsson, Ó.; Thormar, H. In vitro inactivation of *Chlamydia trachomatis* by fatty acids and monoglycerides. *Antimicro. Agents Chemother.* **1998**, *42*, 2290−2294.
14. Thormar, H.; Bergsson, G.; Gunnarsson, E.; Georgsson, G,; Witvrouw, M.; Steingrímsson, Ó.; De Clercq, E.; Kristmundsdóttir, T. Hydrogels containing monocaprin have potent microbicidal activities against sexually transmitted viruses and bacteria in vitro. *Sex. Transm. Inf.* **1999**, *75*, 181−185.

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