Faculty of Pharmacy[1] and Institute of Biology[2], University of Iceland, Reykjavik, Iceland

Effects of polysorbates on antiviral and antibacterial activity of monoglyceride in pharmaceutical formulations

T. Ó. Thorgeirsdóttir[1], H.Thormar[2], T. Kristmundsdóttir[1]

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Thórunn Ósk Thorgeirsdóttir, Faculty of Pharmacy, University of Iceland, Hagi, Hofsvallagata 53, IS-107 Reykjavík, Iceland
thoth@hi.is

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Studies of pure fatty acids *in vitro* have shown that medium-chain saturated and long-chain unsaturated fatty acids are highly active against enveloped viruses such as herpes simplex virus type 1 (HSV-1). Their monoglycerides are also highly antiviral and in some instances at a concentration ten times lower than that of the free fatty acids [1]. Monocaprin (1-monoglyceride of capric acid) is one of the most effective monoglycerides and has been shown to be effective against enveloped viruses such as vesicular stomatitis virus, herpes simplex virus (HSV), visna virus and human immunodeficiency virus *in vitro* [2, 3]. Monocaprin has also been found to possess bactericidal activity *in vitro* against bacteria such as *Staphylococcus aureus* and group B *Streptococcus* [4]. Lipids are a part of the innate immune system in humans, for example in lung surfactants [5, 6] and on the skin [7–10]. A topical pharmaceutical formulation containing the lipid monocaprin could therefore be a suitable choice to prevent transmission of pathogens to mucosal membranes or for the treatment of established mucosal or skin infections caused by a virus or a bacterium [2]. Having a structure similar to the lipids of the innate immune system, monocaprin might also be less irritating than the active substances already in use. Previous work with hydrophilic gels containing monocaprin in a concentration of 20 mM have shown more than 100.000 fold inactivation of HSV-2 and HSV-1 [2, 3]. Monocaprin has limited solubility in aqueous solutions but it is important that the microbicidal lipid is solubilised in an acceptable pharmaceutical formulation which can be easily and effectively applied to mucosa or skin areas which can serve as entry sites for infectious agents. Propylene glycol and glycofurol 75 have been found to be suitable solvents for monocaprin in hydrogels with carbomer as carrier [3]. In this work a combination of co-solvent and a polysorbate surfactant was used to increase the solubility of monocaprin in order to use as low a concentration of the co-solvent as possible. It has previously been reported that the microbicidal activity of monocaprin can be affected by the type and amount of excipient used in the formulation [3]. The main emphasis of this work was to evaluate the effect of polysorbates 20 and 40 at different concentrations on the microbicidal activity of monocaprin formulated either in a hydrogel or in a solution, using propylene glycol as co-solvent. Activity of the monocaprin dosage forms against group B *Streptococcus* and HSV-1 were used to evaluate the effects of the surfactant on microbicidal activity. Over 20 different solutions containing different combinations of the co-solvent propylene glycol and polysorbates 20 or 40 in various concentrations were formulated and four hydrogels containing propylene glycol in either 5 or 7.5% concentration with 0.2 or 0.4% polysorbate 40 were also formulated and compared to the solutions. Table 1 shows that for monocaprin solutions containing 5% propylene glycol the antiviral activity is greatly reduced by 5% polysorbate 20. Solutions containing 7.5% propylene glycol and polysorbate 20 in the concentration range from 0.75 to 1.5% were found to have antiviral activities comparable to that of pure monocaprin (5 $\log_{10}$) but the bactericidal activity decreased with increasing concentration of the solubilizing agent (Table 2). In solutions containing either 5% or 7.5% propylene glycol and polysorbate 40 in the concentration range from 0.1 to 0.4% the microbicidal activity of monocaprin was not affected by the amount of the surfactant (data not shown). A small reduction in bactericidal activity with increasing amount of polysorbate 40 along with 5% propylene glycol is evident after storage for one year (7.5 $\log_{10}$ to 5.6 $\log_{10}$ reduction). Increasing the amount of propylene glycol to 7.5% gives similar results (data not shown). In hydrogels containing either 5% or 7.5% propylene glycol and 0.1 to 0.4% polysorbate 40 the activity of monocaprin against bacteria and virus was not affected by the excipients and the gels showed up to 1 log greater reduction in viral titer (6.0 $\log_{10}$) than the corresponding solutions (5.0 $\log_{10}$). In solutions prepared with 10% propylene glycol neither polysorbate type or concentration in the range previously used affected the antiviral or bactericidal activity of monocaprin. The finding that the antiviral activity of monocaprin solutions decreases with increasing amounts of surfactant suggests that the monoglyceride is trapped in micelles of polysorbate and is not accessible for microbicidal effects. That this effect is seen at lower concentration of polysorbate 40 compared to polysorbate 20 could be explained by its lower CMC value [11]. Increasing the amount of propylene glycol and thus altering the CMC value for the surfactant can also reduce the negative effect and at 10% concentration of the co-solvent the effect is not seen at all. The fact that the antibacterial

Table 1: Antiviral activity of monocaprin solutions containing 5% propylene glycol (PG) as a solvent and different percentage of the solubilizing agent polysorbate 20 (P20)

Polysorbate 20 (%)	Reduction in virus titer ($\log_{10}$) After formulation
1.5	$\geq$5.5
2	$\geq$5
3	$\geq$5
5	1.5

Reduction of virus titer is against herpes simplex virus (HSV-1)

Table 2: Antiviral and antibacterial activity of monocaprin solutions containing 7.5% PG and different percentage of P20. Reduction of virus titer is against HSV-1. Reduction of bacteria titer is against *Streptococcus* group B

Polysorbate 20 (%)	Reduction in virus titer ($\log_{10}$)	Reduction in bacteria titer ($\log_{10}$)
0.75	$\geq$5	$\geq$6.2
1	$\geq$5	$\geq$5.8
1.25	$\geq$5	2.3
1.5	$\geq$5	1.7

activity of monocaprin is more susceptible to an increasing amount of solubilizing agent than the antiviral activity, can possibly be explained by the difference between the simple lipid envelope of the virus and the cell wall of the Gram-positive bacteria. Thus the amount and type of surfactant used in solutions can affect both the virucidal and bactericidal activity of monocaprin. The microbicidal activity of monocaprin hydrogels was neither affected by the co-solvent nor the surfactant concentration used. This difference might be explained by bond formation between the liquid phase and the hydrogel structure, probably with participation of the solubilizing agent. This would result in freely dissolved monocaprin without any micellar entrapment. Another possibility is that the hydrogel structure forms bonds with the viral envelope and the bacterial cell wall, thereby further increasing the contact between the microbicide and the microbe. This is indicated by the results which show that even though the negative effect of the solubilizing agent is overcome by increasing the amount of solvent, solutions always show less antiviral activity than the comparable hydrogels.

Experimental

1. Materials

Propylene glycol and polysorbate 20 and 40 were purchased from Sigma Chemical Co., St. Louis, U.S.A. Monocaprin was obtained from Danisco Ingredients, Denmark, and Carbomer 934 from BFGoodrich. Preservatives and HPMC (hydroxypropyl-metylcellulose) were purchased from NMD, Norway.

2. Preparation of solutions

In preparing the solutions, monocaprin and the preservatives were dissolved in propylene glycol. When they were fully dissolved the solubilizing agent, polysorbate, was added. After adding water the pH was adjusted to 7.0.

3. Preparation of hydrogels

In preparing the hydrogels the gelling agents were allowed to swell in part of the water before gently stirring a solution of propylene glycol, polysorbat, monocaprin and preservatives into the gel, then water was added and pH adjusted to 5.0.

4. Assay of virucidal activity

A volume of 100 µl of herpes simplex virus type 1 (HSV-1) was mixed with an equal volume of gel/solution for 1 min at room temperature. Virus mixed with culture medium served as a control. The mixtures were diluted in culture medium and titrated in tenfold dilutions. The titre ($\log_{10}$) of gel/solution-virus mixture was subtracted from the titre ($\log_{10}$) 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 gel/solution.

5. Assay of bactericidal activity

Clinical isolates obtained from the University Hospital of Iceland were used. A volume of 200 µl of Streptococcus group B was mixed with an equal volume of gel/solution for 10 min at 37 °C. Bacteria mixed with appropriate broth served as a control. The mixtures were diluted in saline and titrated in tenfold dilutions. The titre ($\log_{10}$) of gel/solution-bacteria mixture was subtracted from the titre ($\log_{10}$) of the control mixture and the difference, i.e. the reduction in bacterial infectivity, was used as a measure of the bactericidal activity of the gel/solution.

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