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Development of a virucidal cream containing the monoglyceride monocaprin

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Received May 27, 2005, accepted June 7, 2005

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Pharmazie 60: 897–899 (2005)

The lipid monocaprin (1-monoglyceride of capric acid) has been shown to be effective against enveloped viruses such as herpes simplex virus HSV *in vitro*. As it is known that HSV can develop resistance to acyclovir which is the most common treatment used, it was considered to be of interest to formulate a cream containing the lipid monocaprin as the active substance against HSV. The aim of this study was to develop an o/w-emulsion (cream) containing monocaprin and to evaluate the effects of formulation variables on the virucidal activity of monocaprin as well as the *in vitro* release rate of the monoglyceride from the formulations. The results show that release rate and extent of monocaprin release as well as the microbicidal properties of the the o/w-emulsion formulations are affected by the proportion of the oil phase and the amount of carbomer in the aqueous phase. Reducing the oil volume fraction increased antiviral effect and release of monocaprin from the formulation.

1. Introduction

Herpes simplex virus (HSV) is a virus of the herpesviridae family. HSV must contact mucosal surfaces or abraded skin to initiate infection but the virus can cause a range of diseases from mild uncomplicated mucocutaneous infections to life threatening diseases. The most common sites of HSV infection are skin and mucosal membranes. Acyclovir and its derivatives given orally or intravenously are the standard treatment of serious HSV infections. Topical acyclovir therapy is the most common treatment of the recurrent orolabial lesions that most often are caused by HSV-1 (Whitley and Roizman 2001).
The lipid monocaprin (1-monoglyceride of capric acid) has been shown to be effective against enveloped viruses such as vesicular stomatitis virus, herpes simplex virus and human immunodeficiency virus *in vitro* (Thormar et al. 1987, 1999). Monocaprin has also been found to possess bactericidal activity *in vitro* against bacteria such as *Staphylococcus aureus* and *Group B Streptococcus* (Bergsson et al. 2002). As it is known that HSV can develop resistance to acyclovir (Whitley and Roizman 2001) it was considered to be of interest to formulate a cream containing the lipid monocaprin as the active substance against HSV.
The aim of this study was to develop an o/w-emulsion (cream) containing monocaprin and evaluate the effects of formulation variables on the virucidal activity of monocaprin as well as the release of the monoglyceride from the formulations. Previous work has shown that the microbicidal activity of monocaprin can be reduced by excipients used in the formulation (Kristmundsdóttir et al. 1999; Thorgeirsdóttir et al. 2003). As monocaprin has limited solubility in water it was solubilized using a combination of the co-solvent propylene glycol and the surfactant polysorbate 20. Since earlier work had indicated that the stability of monocaprin was improved by the presence of carbomer, carbomer was included in the aqueous phase (Thorgeirsdóttir et al. 2005). The formulation variables in the cream formulations were: oil volume fraction, amount of carbomer in the aqueous phase and amount of monocaprin (Table 1). To evaluate virucidal activity, all creams were tested against herpes simplex virus type 1 (HSV-1).

Table 1: Formulation variables and HSV-1 activity studies

Formulation	Carbomer in aqueous phase (%)	Volume fraction of oil phase (%)	Monocaprin concentration (mM)	Reduction in virus titer (log_{10}) after 10 min incubation	Reduction in virus titer (log_{10}) after 30 min incubation
1	0.5	2.5	20	4.7	4.5
2	0.5	2.5	0	3.0	3.8
3	0.5	5.0	20	4.7	4.5
4	0.5	5.0	0	3.2	3.8
5	0.5	7.5	20	2.7	4.2
6	0.5	7.5	30	3.5	4.5
7	0.5	7.5	40	3.5	4.5
8	0.5	7.5	0	2.0	2.8
9	0.5	10	20	3.2	3.5
10	0.5	10	0	2.0	2.8
11	0.33	10	20	2.7	3.5
12	0.33	10	0	1.2	2.8

2. Investigations, results and discussion

2.1. Antiviral activity studies

In the formulations monocaprin was contained in the aqueous phase as preliminary studies on cream formulations showed that including the monoglyceride in the oil phase

resulted in loss of virucidal activity. All the cream formulations containing monocaprin did show activity against HSV-1 (Table 1), with a longer incubation time in most cases resulting in a further reduction in virus titer. For formulations containing 5% or lower volume fraction of oil phase the microbicidal activity of the creams was only slightly lower than that of monocaprin in culture medium ($\geq$5.0 log) (Thormar et al. 1987), but the antiviral activity of these creams decreased with increasing volume fraction of the oil phase. These results are in accordance with the preliminary studies mentioned above and what is likely happening is that the lipid monocaprin partitions into the oil phase of the cream and is not easily released from it. Notably, creams without monocaprin showed a significant activity against HSV-1, possibly caused by carbomer. Increase in monocaprin concentration from 20 mM to 40 mM did not increase the virucidal activity of the creams.

2.2. Release of monocaprin from o/w creams

The results show that the release rate of monocaprin from the creams is influenced both by carbomer concentration in the aqueous phase and the volume fraction of the oil phase. Lowering the carbomer concentration in the aqueous phase from 0.5% to 0.33% leads to an increase in total release of monocaprin. The total release of monocaprin increases with reduction of the volume fraction of the oil phase and is greatest for the formulation where the volume fraction of the oil phase is 2.5% (Figs. 1 and 2). All other formulations exhibited a total release of monocaprin around 20% compared to 50% for the one containing 2.5% volume fraction of the oil phase. It could be expected that the lipid monocaprin partitions into the oil phase of the cream and is not easily released from there. The lower oil phase volume also leads to a lowering of cream viscosity and hence there is a faster release of the monoglyceride from the formulation. As is evident from Fig. 2 there is an almost linear relationship between the release rate and the oil volume fraction until the oil volume fraction is reduced to 2.5%. The release of solubilized drug from o/w-emulsion-type creams depends on several factors such as the diffusion coefficient of the drug in the external phase, the partition coefficient between the external and internal phases and the volume fraction of the internal phase (Martin 1993). The combination of these factors affecting the diffusion of monocaprin from the oil phase of the cream to the water phase of the cream and from the water phase of the cream to the receiver phase explains why there can be such a shift.

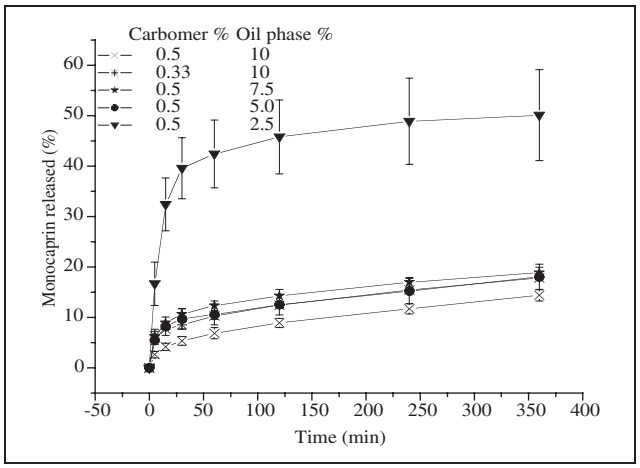

Fig. 1: Release profile of monocaprin from 5 different cream formulations (% of carbomer in aqueous phase/oil volume fraction)

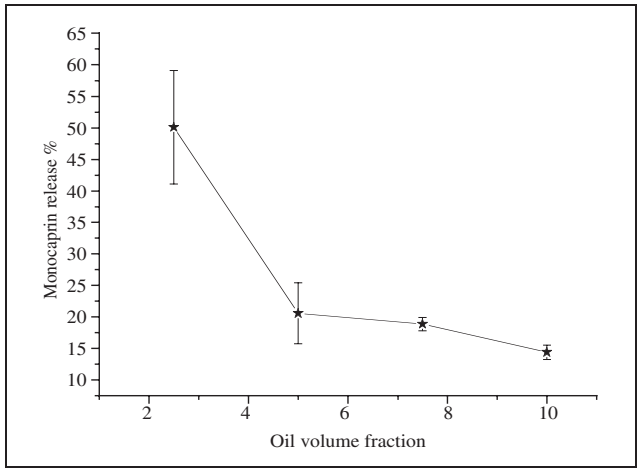

Fig. 2: Percent release of monocaprin versus oil volume fraction

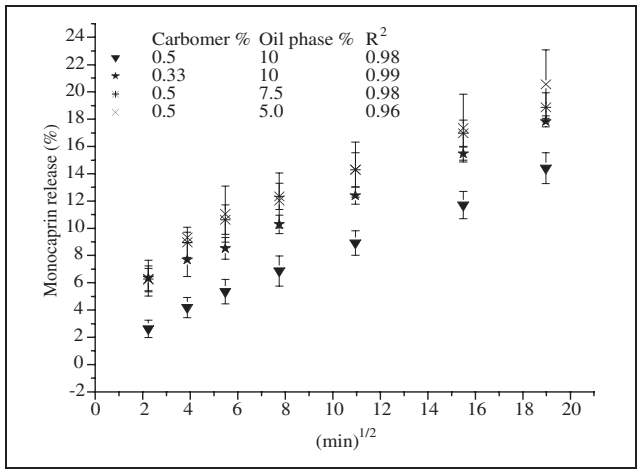

Fig. 3: Percent release of monocaprin from 4 different cream formulations versus square root of time

The data were then analysed with a simplified Higuchi equation which is valid for four of the creams as they exhibit drug release under 30%. The results are expressed in Fig. 3 where the percentage release of monocaprin plotted versus the square root of time gave a straight line. This suggests that the data followed the criteria of Higuchi's equation (Babar et al. 1991; Martin 1993).

In conclusion, the results show that *in vitro* release rate and extent of release of the active ingredient, monocaprin, as well as the microbicidal properties of the the o/w-emulsion formulations are affected by the proportion of the oil phase and the amount of carbomer in the aqueous phase. Reducing the oil volume fraction increased the antiviral effect and the release of monocaprin from the formulation. The formulation where the volume fraction of the oil phase was 7.5% was found to have high virucidal activity as well as acceptable consistency and could be suitable for topical use. Monocaprin was found to be stable in the formulations for six months at 4 °C.

3. Experimental

3.1. Materials

Propylene glycol and polysorbate 20 were purchased from Sigma Chemical Co., St. Louis, U.S.A. Monocaprin (pharmaceutical grade) was a gift

from Danisco Ingredients, Copenhagen, Denmark. Methylparahydroxybenzoate, propylparahydroxybenzoate, cetostearyl alcohol emulsifying vax and paraffinum liquidum were purchased from NMD, Norway. Carbomer 974P was obtained from BFGoodrich.

3.2. Preparation of o/w creams

The oil phase consisted of equal amounts of paraffinum liquidum and cetylan, with the total amount presented in the Table. The aqueous phase contained 5% propylene glycol, 1% polysorbate 20 and variable amounts of carbomer. Monocaprin and preservatives were dissolved in propylene glycol and then 1% polysorbate 20 was added and mixed carefully. Carbomer 974P was allowed to swell in part of the water before gently stirring the propylene glycol, polysorbate 20, preservatives and monocaprin into the gel. Cetostearyl alcohol emulsifying vax was dissolved in paraffinum liquidum at 60 °C. The aqueous phase was then heated to 40 °C and when the oil phase had cooled down to 40 °C the two phases were mixed using a homogenizer.

3.3. Assay of virucidal activity

A volume of 100 µl of herpes simplex virus type 1 (HSV-1) was mixed with an equal volume of cream for 10 or 30 min at room temperature. Virus mixed with culture medium served as control. The mixtures were diluted in culture medium and titrated in tenfold dilutions. The titre ($\log_{10}$) of a cream-virus mixture was subtracted from the titre ($\log_{10}$) of the control and the difference, i.e. the reduction in viral infectivity, was used as a measure of the virucidal activity of the cream.

3.4. Quantitative analysis of monocaprin

The monoglyceride content of the creams was determined using a HPLC component system consisting of a Thermo Separations Products Spectra Series P200 HPLC solvent delivery system, a Cosmosil C18 (4.6×150 mm) column, a Phenomenex C18, 4×3.0 (L × ID) Security Guard, a Hitachi type L-7200 Autosampler, a Thermo Separations Products SP4400 Integrator, and a Thermo Separations Products Spectra Series UV150 detector. The wavelength was 208 nm, and the mobile phase consisted of acetonitrile and water (58 : 42) with the retention time being 4.00 min at 1.5 mL/min flow rate.

3.5. Drug release

Release of monoglyceride was investigated using a membraneless diffusion cell at 37 °C. Phosphate buffer (pH = 4.5) containing 1.25% 2-hydroxypropyl-β-cyclodextrin was used as the receiver phase. Samples were taken from the receiver phase at regular intervals and 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.

Acknowledgement: This work was supported by grants from the Research Fund of the University of Iceland and the Icelandic Research Council, Students fund.

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