Drug formulations intended to stop the spreading of Sexually Transmitted Diseases (STDs)

C. Pedersen¹, A. Slepenkin², E. Peterson² and C. A. S. Bergström¹

¹ Department of Pharmacy, Uppsala University, Uppsala, SE-751 23, Sweden.
² Department of Pathology, University of California, Irvine, CA 92697-480, USA.
Christian.Pedersen@farmaci.uu.se

ABSTRACT SUMMARY

The salicylidene acylhydrazide (SA) compound INP0341 has been formulated as a clinically relevant vaginal gel formulation to be used to prevent spreading of STDs. The solubility enhancers Cremophor and PEG 400 used in low concentrations resulted in a >300-fold solubility increase and enabled the target concentration of 1 mM INP0341 in the gel. Gels based on mucoadhesive polymers (Carbopol 974P NF and Polycarbophil) showed suitable rheology based on a rheological model of squeezing flow. In vitro drug release experiment shows sustained release of INP0341 from the gel with 20% of the dose being released after 24h.

INTRODUCTION

A group of SA compounds has exhibited promising microbicidal properties in a number of recent studies. In an in vitro anti-HIV-1 study, the compounds exhibited IC₅₀ values (50% inhibition of virus) as low as 1 µM, while at the same time being promising from a toxicological point of view¹. The SA compounds have also showed promising results in in vivo studies of protection against Chlamydia and Herpes Simplex, using a mouse model². In the studies mentioned above, the drugs were formulated as solutions of high DMSO concentration.

The main objective of the current project is to formulate the SA compound INP0341 as a clinically relevant gel formulation, suitable for vaginal administration in humans. The gels should solubilize 1 mM INP0341, and they should have suitable rheology ensuring rapid and high coverage of the vaginal tissue and at the same time long contact times. Ideally, the gel should also release INP0341 during at least 24h in vivo.

EXPERIMENTAL METHODS

Gel production. Gels were produced consisting of 1 mM INP0341, 2.0 wt% Cremophor ELP, 0.5 wt% PEG 400, 5.0 wt% glycerol and 1.0-2.0 wt% polymer. The polymers were Carbopol 974P NF (Lubrizol) and Polycarbophil (Lubrizol), used at a ratio of 1:2. The pH of the gels was adjusted to 5.2.

Rheology. Rheological measurements were performed using a Bohlin VOR controlled rate rheometer (Bohlin Reologi). Shear stress was determined by rotational viscometric measurements, at shear rates 1.16 - 73.2 s⁻¹. Data were collected over a sweep of lowest to highest shear rate, and results are reported as the mean and standard deviation of 3 measurements. The gels exhibited yield stresses, and it was therefore suitable to fit shear stress data to a Herschel-Bulkley constitutive equation³

\[
\sigma = \sigma_y + m(\gamma)^n
\]  

where \(\sigma\) is the shear stress, \(\sigma_y\) is the yield stress, \(m\) is the consistency index, \(\gamma\) is the shear rate and \(n\) is the shear-thinning index. The parameters were determined by fitting experimental data to the model and using the method of least squares.

To obtain an estimate for the gel spreading rate over vaginal mucosa, and to support the choice of polymer concentrations, a theoretical squeezing flow model was employed³.

In vitro drug release. 9 mm Franz cells with 5 ml receptor volume were used to study in vitro drug release from gels. 0.50 ml gel was applied to the donor compartment, and Simulated Vaginal Fluid (SVF)⁴ was used as receptor liquid. A 5.0 µm polycarbonate membrane (Whatman) was used, and the experiments were performed at 37°C. Results are presented as the
mean and standard deviation of 3 measurements. Samples were taken at 1, 2, 3, 4, 5, 6 and 24h, and analyzed with LC-MS.

RESULTS AND DISCUSSION

All carbopol-based gels exhibited yield stresses, which is typical of gels based on poly(acrylic acid)-polymers such as Carbopol and Polycarbophil. Yield stress is in general a beneficial property of vaginal gels, since it decreases the risk of gel leakage from the vagina. The shear stresses of the gels, as function of shear rate and polymer concentration, are shown in Figure 1. The experimental values in Figure 1 were fitted to Eq.1 to obtain the parameters yield stress, consistency index and shear-thinning index. These parameters were then used to obtain estimates for the gel spreading rates over the vaginal mucosa, presented in Table 1.

Figure 1. Shear stress of gels with 1-2 wt% polymer (Carbopol 974P NF:Polycarbophil at 1:2 ratio). The symbols are experimental data, and the lines represent fitting to Eq. 1.

The gel containing 1.5 wt% CP was chosen as suitable for in vivo use, and therefore investigated regarding in vitro drug release. The gel exhibited a linear release profile over a 24h period, and 20.2 ± 4.9 % of the INP0341 dose had been released after 24h. The formulation is currently investigated for in vivo protection against Chlamydia in a mouse model and will at a later stage be tested for protection against HIV.

Table 1. Prediction of vaginal surface area covered by gel 120 min after administration. A mathematical simulation of squeezing flow was used. Data in normal font are based on experimental values obtained (at 37°C) in the present study, while data in italic are based on literature values (obtained at 24°C).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Part of vaginal area covered after 120 min (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00 wt% CP</td>
<td>105</td>
</tr>
<tr>
<td>1.25 wt% CP</td>
<td>95</td>
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<tr>
<td>1.50 wt% CP</td>
<td>83</td>
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<tr>
<td>1.75 wt% CP</td>
<td>76</td>
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<tr>
<td>2.00 wt% CP</td>
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<tr>
<td>Crinone</td>
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<tr>
<td>Replens</td>
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<td>KY Plus</td>
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<tr>
<td>Advantage</td>
<td>94</td>
</tr>
</tbody>
</table>

CONCLUSION

An INP0341 gel formulation (1.5 wt% CP) suitable for in vivo use has been developed. Low concentrations of the solubility enhancers Cremophor and PEG 400 enabled an INP0341 concentration of 1 mM to be reached. Suitable rheology has been ensured via a theoretical prediction of vaginal surface area coverage. A sustained release of INP0341 from the gel was observed, and roughly 20 % of the dose had been released after 24h.

REFERENCES


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