Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 256 (2003) 123–131
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

5-Fluorouracil in vesicular phospholipid gels for anticancer treatment: entrapment and release properties

N. Kaiser [a,*], A. Kimpfler [a], U. Massing [b], A.M. Burger [b], H.H. Fiebig [b], M. Brandl [c], R. Schubert [a]

[a] *Department of Pharmaceutical Technology, Albert-Ludwigs-University of Freiburg, Hermann-Herder-Str. 9, D-79104 Freiburg, Germany*
[b] *Department of Clinical Research, Tumor Biology Center, Breisacher Str. 117, D-79106 Freiburg, Germany*
[c] *Department of Pharmaceutics and Biopharmaceutics, Institute of Pharmacy, University of Tromsø, N-9037 Tromsø, Norway*

Received 21 July 2002; received in revised form 27 November 2002; accepted 19 December 2002

Abstract

Vesicular phospholipid gels (VPG), i.e. highly concentrated liposomal dispersions, are suitable for entrapping substances such as anticancer drugs with particular high encapsulation efficiencies (EE). We prepared different formulations of VPG with 30% (w/w) lipid containing 5-fluorouracil (5-FU) by high pressure homogenization and analysed their EE and drug release. Using mixtures of hydrogenated soy phosphatidylcholine and cholesterol with molar ratios ranging from 55/45 to 75/25, a decreasing amount of cholesterol correlated with an increasing EE, which is probably due to a reduced amount of smaller vesicles and number of lamellae. Using a 5-FU solution of pH 8.6 for VPG preparation, an EE of approximately 40% was found after redispersion of the gel to a liposomal dispersion and separation of free drug from liposomal drug by size exclusion chromatography. The reduced EE for preparations with lower pH values was attributed to a fast initial drug release due to the increased drug lipophilicity below the pK_a value of 8. After redispersion of a VPG of pH 8.0, an initially faster release of about a third of the entrapped drug was found during the first 20 min, followed by stable entrapment over many hours. The rapid initial release may be due to the portion of liposomes smaller than 40 nm in diameter, determined by photon correlation spectroscopy. Cryo electron microscopic pictures show a lentil-like shape of these small liposomes. The membrane defects on the edges are probably the reason for the very high initial drug release rate. The half-life time of the release of 5-FU from intact FU-VPG at both pH 7.4 and 8.0 was found to be in the order of 4–5 h and the kinetics are typical for matrix-controlled drug diffusion. The in vitro data of 5-FU loaded VPG suggest their applicability as implants with controlled release properties or, after redispersion, as intravenously injected liposomal formulations.

Keywords: 5-Fluorouracil; Vesicular phospholipid gel; Liposomes; In vitro studies

1. Introduction

For more than four decades the antineoplastic agent 5-fluorouracil (5-FU) has been used in the therapy of different solid tumor types such as cancer of the gastrointestinal tract (stomach, colon, rectum) and breast cancer. Because of the short plasma half-life of 10–20 min, high doses, e.g. 400–600 mg/m^2 weekly, have to be administered to reach therapeutic drug

Abbreviations: 5-FU, 5-fluorouracil; HSPC, hydrogenated soy phosphatidylcholine; Chol, cholesterol; VPG, vesicular phospholipid gel; FU-VPG, 5-FU-containing vesicular phospholipid gel; EE, encapsulation efficiency; DPPC, dipalmitoylphosphatidylcholine; PS, phosphatidylserine

* Corresponding author. Tel.: +49-761-203-6365/37; fax: +49-761-203-6366.
E-mail address: nicole.kaiser@pharmazie.uni-freiburg.de (N. Kaiser).

0378-5173/03/$ – see front matter
doi:10.1016/S0378-5173(03)00069-3

levels (Peters et al., 1993; Holland et al., 1997). Moreover, the clinical use of 5-FU is limited by its gastrointestinal toxicity (stomatitis) and myelotoxicity (Fraile et al., 1980).

Several efforts have been made to reduce these side effects by encapsulation of 5-FU in liposomes (Mazumder, 1981; Özer, 1992; Elorza et al., 1993; Fresta et al., 1993). However, when using conventional liposome preparation methods, the trapping efficiency of a water-soluble compound such as 5-FU, which is non-interacting with the bilayer (Tsukada et al., 1984) is low. This effect particularly applies to small liposomes due to the small ratio of trapped to external volume. For 5-FU the trapping efficiency is reported to be only 2–6%, depending on the lipid composition (Özer, 1992; Fresta et al., 1993). Another problem is the more or less rapid leakage of the low-molecular 5-FU out of the liposomes after removal of the non-entrapped material (Özer, 1992; Elorza et al., 1993; Fresta et al., 1993) which limits the storage time of conventional liposomal preparations.

Encapsulation efficiency (EE) as well as shelf-life of liposomal formulations can be dramatically enhanced by entrapment of a drug such as 5-FU into vesicular phospholipid gels (VPG) (Brandl and Reszka, 1995; Brandl et al., 1997a, 1997b). VPG are semisolid matrices of densely packed liposomes, mainly small unilamellar vesicles, that are prepared by high-pressure homogenization (Brandl et al., 1990, 1993). Due to their high lipid content, which leads to a considerably increased ratio of aqueous volume inside the vesicles in comparison with the surrounding aqueous volume, these formulations are suitable for entrapping water-soluble substances with a high EE up to over 50% (Brandl et al., 1998). Therefore, there is no need to remove the non-encapsulated drug (Brandl and Reszka, 1995; Brandl et al., 1997a, 1997b). The constant ratio of encapsulated to free drug leads to an increased shelf-life of VPG compared to conventional liposomal formulations as shown for VPG formulations with the anticancer drugs gemcitabine (Moog, 1998; Moog et al., 2002) or vincristin (Güthlein, 2001).

In this study, we analysed the main in vitro characteristics, EE and 5-FU-release of different preparations of VPG with 5-FU with regard to the desired application, intratumorally as semisolid VPG or intravenously after redispersion to injectable liposomal formulation.

2. Materials and methods

2.1. Materials

Hydrogenated soy phosphatidylcholine (HSPC) was a generous gift from Lipoid (Ludwigshafen, Germany). Cholesterol was obtained from Caelo (Caesar & Lorentz, Hilden, Germany), 5-FU from Fluka laboratory chemicals (Fluka, Taufkirchen, Germany) and Sepharose CL-4B from Pharmacia (Uppsala, Sweden). All other materials and solvents were of analytical grade.

2.2. Methods

2.2.1. Preparation of VPG

2.2.1.1. Empty VPG. VPG consisted of Chol and HSPC (different molar ratios 25/75 up to 45/55) with a total lipid content of 30% (w/w) (up to approximately 500 mM total lipid in the case of maximum Chol) or 40% (w/w) (up to approximately 665 mM total lipid) in isotonic phosphate buffer (50 mM, adjusted to the desired pH) as described previously (Brandl et al., 1990, 1993; Brandl and Reszka, 1995). In brief: HSPC and Chol were dissolved in chloroform/methanol (2:1 v/v). The solvent was removed under reduced pressure at 40 °C (rotatory evaporator) resulting in a thin lipid film. Solvent traces were completely removed by vacuum for 24 h. The dry lipid film was hydrated for 3 h using an appropriate volume of isotonic phosphate buffer. The resulting semisolid lipid dispersion was homogenized 10 times with high pressure (70 MPa) in a discontinuously working apparatus (APV Gaulin Micron Lab 40, APV, Unna, Germany). The resulting VPG was autoclaved (121 °C, 15 min) and stored until use at 4–8 °C (Tardi, 1999).

2.2.1.2. Photon correlation spectroscopy. For size determination, VPG were first redispersed and then diluted with freshly filtrated PBS buffer (0.22 μm Minisart, Sartorius AG, Göttingen, Germany) in order to reach a count rate of 250–350 kHz, which is recommended by the manufacturer of the PCS

instrument (Nicomp submicron particle sizer model 380 ZLS, Nicomp Inst Corp., Santa Barbara, CA). All samples were placed in the machine for 5 min prior to the start of the measurement to eliminate temperature differences between the sample and the instrument. Three cycles of 5 min each were run for data collection. The instrument parameters were set as follows: automatic choice of channel width; vesicle mode; number weighting; automatic change from Gaussian distribution mode to multimodal mode (so-called Nicomp distribution mode) if the value for Chi-squared exceeded 3.00.

2.2.1.3. Cryotransmission electron microscopy. Copper grids (200 mesh, Science Services, Munich, Germany) were prepared according to a standard method as described elsewhere (Fukami and Adachi, 1965). After pulling a drop of the sample on a grid, most of the liquid was removed with blotting paper leaving a thin film stretched over the wholes. The specimens were instantly shock-frozen by plunging into liquid ethane, cooled to 90 K by liquid nitrogen in a temperature controlled freezing unit (Zeiss, Oberkochen, Germany). After removal of the remaining ethane, the specimens were transferred to the microscope using a cryo holder (Oxford, Abingdon, UK). The transmission electron microscope was a Leo 912 Omega (Zeiss, Oberkochen, Germany), equipped with a cryo stage. Thus, examinations could be carried out at low temperatures of approximately 100 K. Zero-loss filtered images ($\Delta E = 0$ eV) were taken by a SSCCD camera, under low-dose conditions, i.e. using the minimal dose focusing device.

2.2.1.4. Loading techniques. There were two possibilities for loading 5-FU into the VPG. Using the *passive loading technique* (Massing et al., 1998), known amounts of empty VPG containing 40% total lipid were transferred into 2 ml reaction vials under sterile conditions. Appropriate volumes of 5-FU stock solution (4.2 mg/ml 5-FU in PBS buffer adjusted to pH 7.4) were added and the components were mixed thoroughly by using sterile plastic spatulas. The resulting VPG had a total lipid amount of 30% (w/w) and the mixtures were incubated at 60 °C for several hours to facilitate the diffusion of the 5-FU into the liposomes. To achieve *direct entrapment* the FU-VPG is prepared in a similar way to the empty VPG but by hydrating the lipid film with a 5-FU containing buffer solution (either 4.2 mg/ml 5-FU in PBS buffer adjusted to pH 7.4 or 12.4 mg/ml 5-FU in PBS buffer adjusted to pH 8.0 rsp. 8.6) for 3 h prior to homogenization.

2.2.2. Redispersion of VPG

In a reaction vial six sterile glass beads (diameter 1 mm) and 675 mg of the VPG were diluted with PBS buffer (ratio of VPG/buffer 1:1.2 w/w). The VPG was redispersed to a liposome dispersion using a ball mill (Retsch MM2, Haan, Germany) for 2 min at 2000 rpm (Brandl and Reszka, 1995).

2.2.3. Encapsulation efficiency

Separation of liposomal from free 5-FU was performed on a Sepharose CL-4B column (0.7 cm × 10 cm). For this purpose, the liposome dispersion was diluted with buffer to a concentration of 30 mM lipid at the maximum. Fifty microliters of this dispersion were placed gently on top of the column. Liposomally encapsulated 5-FU was separated from non-entrapped (free) 5-FU by elution with buffer and quantified as described below. EE was calculated by:

$$\mathrm{EE}(\%) = c_{\mathrm{lip}} \times \frac{100}{c_{\mathrm{lip}} + c_{\mathrm{free}}}$$

where c_{lip} is the concentration of liposomally entrapped 5-FU, and c_{free} is the concentration of free 5-FU.

2.2.4. Determination of 5-FU

Concentrations of 5-FU were determined by HPLC. When necessary, the samples were diluted with HPLC eluent and the 5-FU amount was quantified by a modified method described elsewhere (Del Nozal et al., 1994). The HPLC consisted of a SunFlow 100 solvent delivery pump (SunChrom GmbH, Friedrichsdorf; Germany), a Basic-Marathon Plus autosampler (Spark Holland B.V., Emmen, The Netherlands) with a 50 μl loop, a PU 4110 UV/VIS-detector (Unicam Chromatography, Cambridge, UK) operating at 265 nm and the chromatography data handling system Unicam 4880 Version 1 including an PU 6030 DCU data interface. Chromatography was carried out on a 5 μm Waters Spherisorb® ODS2 analytical

column (5 μm, 250 mm × 4.6 mm i.d.; ThermoHypersil, Ceshire, UK) using a suitable precolumn. The mobile phase consisted of 50 mM phosphate buffer pH 7.4/MeOH (98/2 v/v) and was run with 1 ml/min. The buffer was filtrated through 0.2 μm cellulose acetate filters (Sartorius AG, Goettingen, Germany) and then mixed with the MeOH. Subsequently, the mobile phase was degassed by ultrasonication under reduced pressure for 10 min before chromatography was carried out at room temperature. Results were calculated from linear regression of an external standard of 5-FU.

2.2.5. Release of 5-FU from redispersed VPG

The release of 5-FU from liposomes after redispersion of VPG was determined via analysis of the trapping efficiency at several time points after redispersion. The VPG was redispersed as described in Section 2.2.2 and the liposomal dispersion was kept at room temperature. After certain points in time, an aliquot was diluted and the EE was determined by column chromatography (see Section 2.2.3).

2.2.6. Release of 5-FU from non-redispersed VPG

The release of 5-FU from semisolid VPG was examined using custom-made flow through cells (Tardi, 1999). The cells consisted of a combined aluminum block with a lower and an upper half. The lower half was right-angled reamed (height 5 mm, width 5 mm, length 50 mm) to obtain a constant contact surface between the elution medium and the VPG during the whole experiment. The upper half (length 50 mm) was half-round reamed (radius 2.5 mm) to achieve a laminar flow of the elution medium over the gel. FU-VPG was centrifuged at 1500 × *g* for 60 min (Biofuge pico, Heraeus, Osterode, Germany) to remove air bubbles issuing from preparation and filled into the lower half which had a carrying capacity of 1 g of preparation. The two halves were locked watertight and the system was allowed to equilibrate in a water bath at 37 °C for 30 min. Isotonic PBS buffer pH 7.4 was used as elution medium flowing over the VPG with a flow rate of 10 ml/h according to the order of magnitude of intraperitoneal and cerebral fluid flow (Cutler et al., 1973; Diem and Lentner, 1977; Hierholzer and Schmidt, 1991). During the release test, the buffer solution and the flow through cells were kept constant at 37 °C. Fractions were collected, and released liposomal and free 5-FU was quantified by HPLC.

3. Results and discussion

3.1. Preparation of VPG

Different formulations of FU-VPG were prepared by varying the pH of the 5-FU stock solution and the molar lipid ratio of the lipid phase consisting of HSPC and Chol. Drug loading into VPG can be performed by either *direct entrapment* or the *passive loading technique* (see Section 2). VPG production by *direct entrapment* is performed by high pressure homogenising a lipid film with a drug containing buffer solution. To ensure sterility for parenteral application, either intratumoral as semisolid VPG or intravenous as redispersed VPG, autoclaving at standard conditions (121 °C, 15 min) after preparation is possible for VPG (Tardi et al., 2001). But this method can only be performed when the particular drug is heat-stable, which is indeed the case for 5-FU (data not shown). However, for every desired drug concentration a separate VPG has to be produced. This problem can be overcome by the passive loading technique, which has the additional advantage of allowing to load less heat-stable drugs (Massing et al., 1998). A disadvantage of this method compared to direct entrapment is the reduction of the 5-FU concentration of the stock solution by a dilution factor of about 4 in the final VPG.

5-FU was shown elsewhere not to adsorb to DPPC/PS bilayers (Fresta et al., 1993). This could be confirmed by own studies, also for liposomes from HSPC and Chol mixtures (data not shown). Consequently, in the VPG a maximum 5-FU concentration corresponding to the drug solubility in the buffer can be entrapped and a high 5-FU dose requires a high VPG volume. In fact the solubility of 5-FU (pK_a, 8 and 13) in water can be increased significantly by increasing the pH (Rudy and Senkowski, 1973). However, taking the stability optimum of the phosphatidylcholine of around pH 6.5 (Grit et al., 1993) into consideration the solubility of 5-FU at pH values suitable for VPG is quite low. For all these reasons the "*passive loading technique*" as used for VPG formulations of the anticancer drugs gemcitabine (Moog, 1998; Moog et al., 2002) and vincristin (Güthlein,

Table 1
PCS analysis of redispersed VPG 30% HSPC/Chol 75/25 ($n = 3$)

1st peak		2nd peak		3rd peak		Parameter		
Diameter (nm)	Amount (%)	Diameter (nm)	Amount (%)	Diameter (nm)	Amount (%)	Fit error	Residual	Data (k)
115	96	481	4	–	–	1.16	41.0	2472
33	55	127	44	680	1	0.63	12.3	5357
33	65	132	35	–	–	0.76	0.0	1477

2001) was not suitable for preparing a VPG formulation with 5-FU. But with 5-FU being sufficiently heat-stable we could overcome these problems by applying *direct entrapment* utilising the total aqueous volume for dissolving 5-FU.

3.2. Size distribution

The vesicle size distribution of redispersed VPG from HSPC/Chol was examined by PCS and cryo electron microscopy. Inhomogeneous sizes distributions were calculated for all preparations when the multimodal analysis was used, indicating at least two size populations (see Table 1). In addition, some very large liposomes of up to 1000 nm could be fitted. To avoid microembolism in the capillaries, it is therefore necessary to remove these before the liposomal dispersion is used for intravascular application. After filtration through 800 nm pores, a loss of lipid of about 2% of the total lipid was found to be negligible. The total EE decreased only from 33 to 31% suggesting the usefulness of this procedure.

Additional information on liposome size and shape after redispersion of the VPG was obtained by cryo EM pictures (see Fig. 1a and b). The broad size distribution confirms the PCS measurements. Furthermore, in contrast to earlier findings of the normal spherical shape of the liposomes redispersed from VPG consisting of fluid lipid mixtures (Brandl et al., 1997a; Brandl et al., 1998), liposomes from HSPC and Chol show a lentil-like shape below a critical diameter of approximately 40 nm. Obviously, the membrane curvature is limited for this rigid lipid mixture and the membranes then form edges. These defects may substantially increase the drug release rate deriving from the portion of these small liposomes.

3.3. Encapsulation efficiency (EE)

3.3.1. Influence of the lipid composition

VPG with different molar ratios of HSPC/Chol of 55/45, 65/35 and 75/25 were prepared by *direct entrapment* with a 5-FU stock solution of pH 7.4. A decrease of the Chol amount from 45 to 25 mol% resulted in a dramatic increase in EE from 8% to approximately 18% (see Table 2a). A possible difference in liposomal drug association due to varying drug adsorption to the membranes was excluded by binding studies (data not shown). No membrane interaction of 5-FU was found, which was comparable to results using other membranes (Fresta et al., 1993). Therefore, the increase in EE upon increasing the HSPC content must be due to an increasing amount of larger liposomes and/or a decrease in liposome lamellarity. However, both characteristics of the redispersed VPG are hardly to quantify by PCS or cryo EM.

Table 2a
Encapsulation efficiency of FU-VPG 30% pH 7.4 depending on the cholesterol content ($n = 3$)

Molar ratio of HSPC/Chol	EE (%)
55/45	8.0 ± 2.4
65/35	10.4 ± 1.5
75/25	18.5 ± 2.5

Table 2b
Encapsulation efficiency of FU-VPG 30% (HSPC/Chol 75/25) depending on the pH of the 5-FU containing stock solution ($n = 3$)

	EE (%)
pH 7.4	18.5 ± 2.5
pH 8.0	33.3 ± 0.3
pH 8.6	39.6 ± 0.9

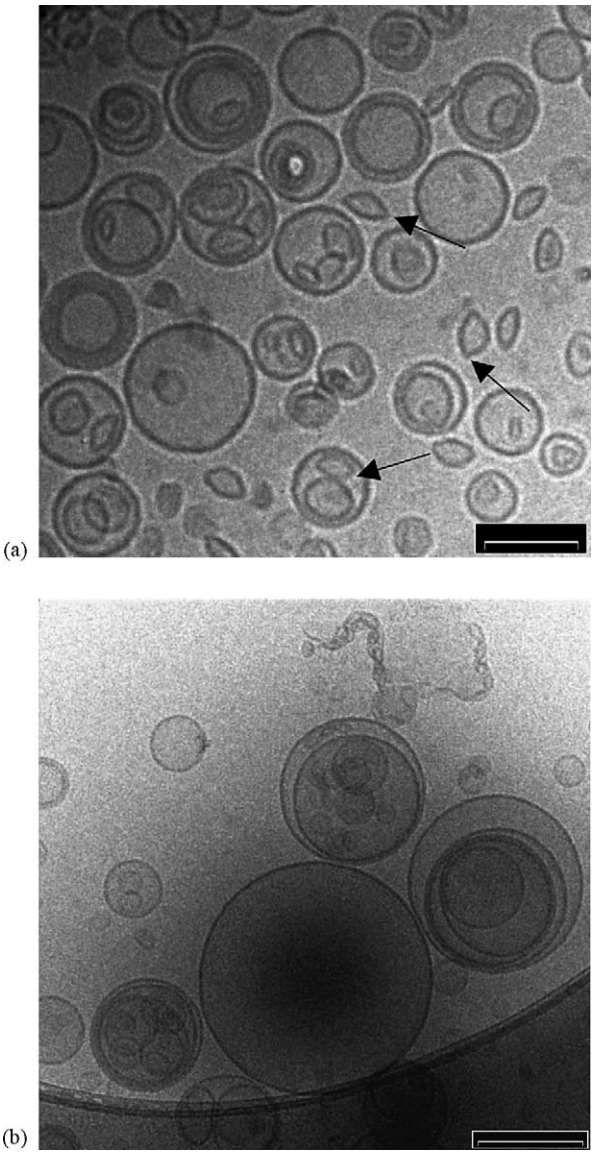

Fig. 1. Electron micrographs of redispersed VPG 30% HSPC/Chol 75/25 pH 8.0 after cryofixation. (a) Lentil-like liposomes below a critical diameter of approximately 40 nm (indicated by arrows) free and entrapped in other vesicles. Bar represents 100 nm. (b) Oligolamellar and multivesicular vesicles beside lentil-like structures forming a chain. Bar represents 200 nm.

3.3.2. *Influence of the pH value*

When the pH of the 5-FU stock solution was changed, the preparations of VPG from HSPC/Chol 75/25 mol/mol exhibited significant changes in EE (see Table 2b). pH-dependent adsorption of 5-FU to the membrane can be excluded from binding studies (see above). Therefore, the different results are obviously influenced by the procedure of EE determination. After redispersing the VPG and separating the liposomal from the free drug, the first time point of measurement was approximately 8 min. During this time different amounts of 5-FU, depending on the pH, leak out. At lower pH, due to higher protonation and lipophilicity of 5-FU, its release is faster pretending a lower EE. The high EE values found for a high pH of 8.6 were comparable to VPG formulations of other cytostatics such as the hydrophilic gemcitabine (dFdC) with approximately 33% (Moog, 1998). For vincristin, which probably adsorbs to the membranes, the EE was found to be approximately 56% (Güthlein, 2001).

However, a high pH for achieving maximum EE is not suitable for longer storage of the VPG. Phospholipids show a maximum stability against hydrolysis at pH 6.5 (Grit et al., 1993). Therefore, a somewhat lower pH of 8.0 with an EE of approximately 33% should be used as a compromise between trapping and stability.

3.4. *Release of 5-FU from redispersed VPG*

Knowledge about drug release from redispersed VPG is important for estimating the suitable time interval between VPG redispersion and application. By redispersing 1 part semisolid VPG (approximately 30% w/w lipid), showing an initial EE of 35% (approximately 25% v/v), with 1.2 parts of buffer, the external aqueous compartment (approximately 45% v/v) is diluted and the EE should end up at an equilibrium value of approximately 13%. Fig. 2 shows that from the VPG pH 8, a fast release of 5-FU occurs within the first 20 min after redispersing, which may be due to the portion of defective liposomes, as discussed above. Thereafter, the permeation through the rigid membranes of the larger liposomes is slower resulting in an EE of approximately 25%, which is after 2 h far from theoretical equilibrium distribution due to low protonation, i.e. high hydrophilicity. In accordance with these considerations redispersion of VPG pH 7.4 resulted in a relatively constant EE of around 13% (equilibrium). This substantiates the very fast release of the lipophilic drug below the pK_a value, as discussed above.

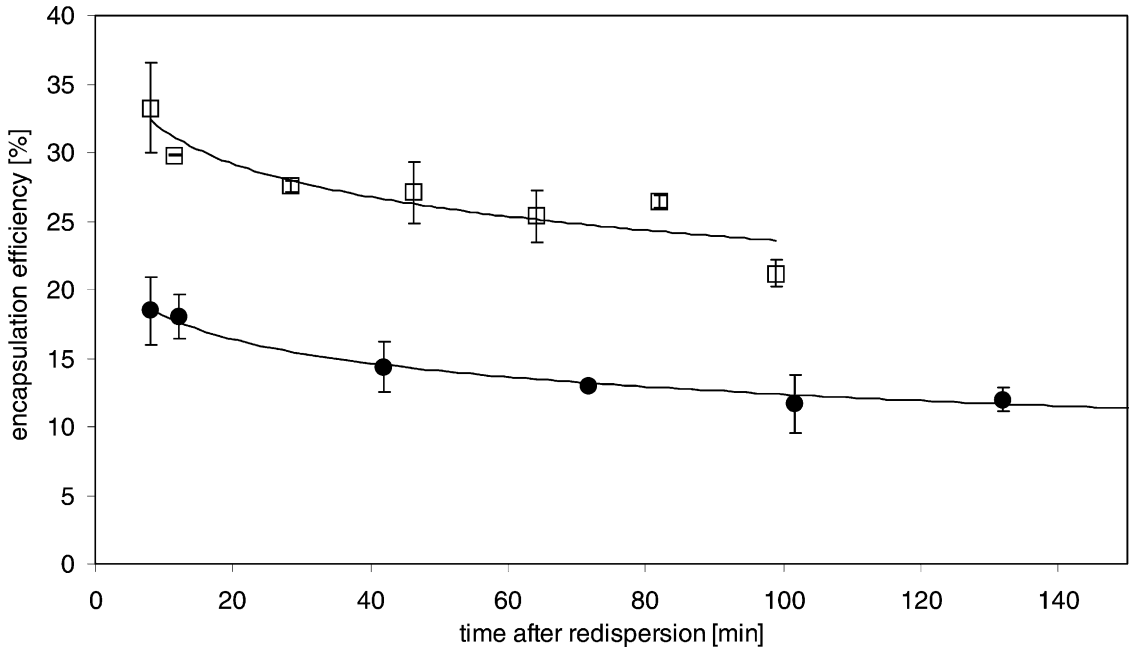

Fig. 2. Encapsulation efficiency of 5-FU after redispersion of FU-VPG 30% (HSPC/Chol 75/25 mol/mol) prepared with FU-stock solutions of two different pH values (mean ± standard deviation; $n = 3$). (●) pH 7.4; (□) pH 8.0.

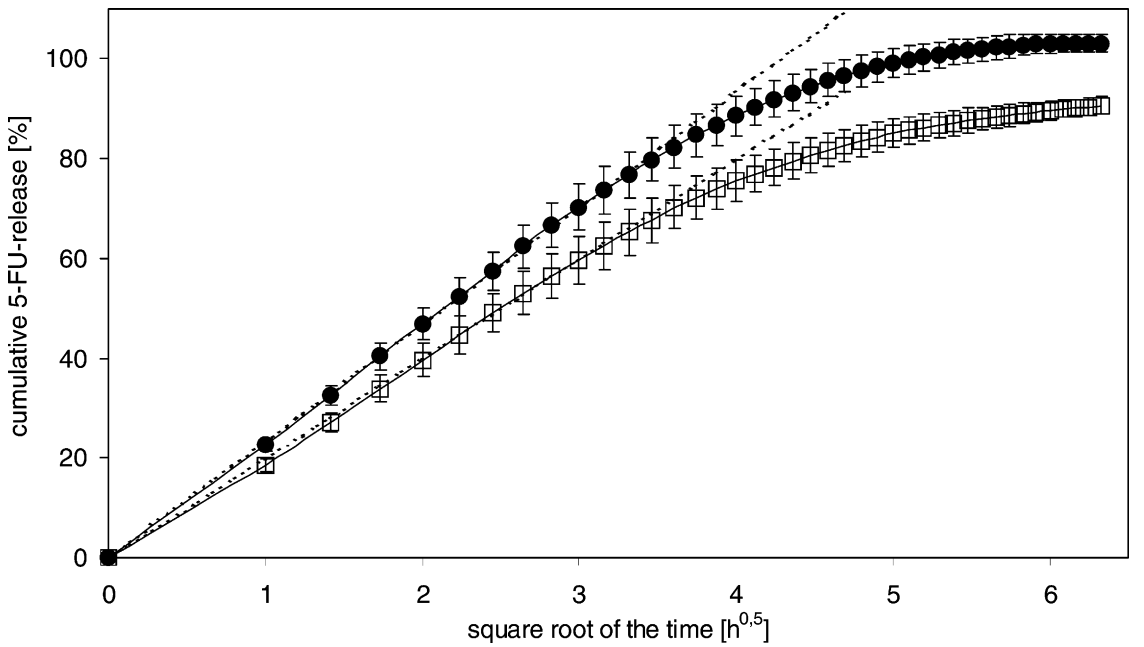

Fig. 3. Cumulative release of 5-FU from FU-VPG 30% (HSPC/Chol 75/25 mol/mol) prepared with FU-stock solutions of different pH values (mean ± standard deviation, $n = 3$) vs. the square root of the time. (●) pH 7.4; (□) pH 8.0. Linear fit of the corresponding curve for the time period of 0–12 h. $r = 0.99957$ for pH 7.4, $r = 0.99910$ for VPG pH 8.0.

3.5. Release of 5-FU from non-redispersed VPG

The release of 5-FU was measured in triplicate from VPG 30% (molar ratio of HSPC/Chol 75/25) with different concentrations of 5-FU: pH 7.4 with 4.2 mg/ml and pH 8.0 with 12.4 mg/ml. 5-FU was completely released from VPG pH 7.4 within 37 h and almost released from VPG pH 8.0 within 100 h (96% release in 100 h). The half-life time was found to be 4.5 h for VPG pH 7.4 and 5.5 h for VPG pH 8.0. As illustrated in Fig. 3, the release of 5-FU could be described as matrix-controlled diffusion kinetics of up to 12 h (square-root-of-time law (Higuchi, 1960); $r = 0.99957$ for VPG pH 7.4; $r = 0.99910$ for VPG pH 8.0).

4. Conclusion

The presented in vitro data of 5-FU in VPGs prepared from HSPC/Chol show a highly reproducible drug release and suggest an applicability of these therapeutic systems (i) as implants in or around solid tumours with controlled release properties or (ii) after redispersion, as intravenously injectable liposomal formulations with prolonged drug release. A pH of 8.0 of the preparations ensures high drug loading even after redispersion of the gel over many hours. Binding studies of 5-FU (data not presented) show no significant adsorption of 5-FU to the liposome membranes. Therefore, the drug release is controlled by the rigidity of the liposome membranes, the occurrence of membrane defects in the very small liposomes and on the pH of the formulation, which determines the drug lipophilicity.

Acknowledgements

The authors would like to thank I. Ohlhoff and S. Linser-Haar for their technical support. This work was supported by the Government of the Federal State of Baden-Württemberg, Zukunftsoffensive “Junge Generation”, grant: 720.430-22/17A.

References

Brandl, M., Bachmann, D., Drechsler, M., Bauer, K.H., 1990. Liposome preparation by a new high pressure homogenizer Gaulin Micron Lab 40. Drug. Dev. Ind. Pharm. 16, 2167–2191.

Brandl, M., Bachmann, D., Drechsler, M., Bauer, K.H., 1993. Liposome preparation using high-pressure homogenizers. In: Gregoriadis, G. (Ed.), Liposome Technology, vol. 1. CRC Press, Boca Raton, pp. 49–65.

Brandl, M., Drechsler, M., Bachmann, D., Bauer, K.H., 1997a. Morphology of semisolid aqueous phosphatidylcholine dispersions, a freeze fracture electron microscopy study. Chem. Phys. Lipids 87, 65–72.

Brandl, M., Drechsler, M., Bachmann, D., Tardi, C., Schmidtgen, M., Bauer, K.H., 1998. Preparation and characterisation of semisolid phospholipid dispersions and dilutions thereof. Int. J. Pharm. 170, 187–199.

Brandl, M., Reszka, R., 1995. Preparation and characterisation of phospholipid membrane gels as depot formulations for potential use as implants. Proc. Int. Symp. Control Release Bioact. Mater. 22, 472–473.

Brandl, M., Tardi, C., Drechsler, M., Bachmann, D., Reszka, R., Bauer, K.H., Schubert, R., 1997b. Three-dimensional liposome networks: freeze fracture electron microscopical evaluation of their structure and in-vitro analysis of release of hydrophilic markers. Adv. Drug. Deliv. Rev. 24, 161–164.

Cutler, R.W.P., Murray, J.E., Moody, R.A., 1973. Overproduction of cerebrospinal fluid in communicating hydrocephalus. A case report. Neurology 23, 41–47.

Del Nozal, M.J., Bernal, J.L., Pampliega, A., Marinero, P., Pozuelo, M., 1994. Determination of the concentrations of 5-fluorouracil and its metabolites in rabbit plasma and tissues by high-performance liquid chromatography. J. Chromatogr. B Biomed. Appl. 656, 397–405.

Diem, K., Lentner, C., 1977. Wissenschaftliche Tabellen, Documenta Geigy, Georg Thieme Verlag, Stuttgart.

Elorza, B., Elorza, M.A., Frutos, G., Chantres, J.R., 1993. Characterization of 5-fluorouracil loaded liposomes prepared by reverse-phase evaporation or freezing–thawing extrusion methods: study of drug release. Biochim. Biophys. Acta 1153, 135–1342.

Fraile, R.J., Baker, L.H., Buroker, T.R., Horwitz, J., Vaitkevicius, V.K., 1980. Pharmacokinetics of 5-fluorouracil administered orally, by rapid intravenous and by slow infusion. Cancer Res. 40, 2223–2228.

Fresta, M., Villari, A., Puglisi, G., Cavallaro, G., 1993. 5-Fluorouracil: various kinds of loaded liposomes: encapsulation efficiency, storage stability and fusogenic properties. Int. J. Pharm. 99, 145–156.

Fukami, A., Adachi, K., 1965. A new method of preparation of a self perforated micro plastic grid and its application. J. Electron. Microsc. 14, 112–118.

Grit, M., Underberg, W.J., Crommelin, D.J., 1993. Hydrolysis of saturated soybean phosphatidylcholine in aqueous liposome dispersions. J. Pharm. Sci. 82, 362–366.

Güthlein, F., 2001. Darstellung und Prüfung von vesikulären Phospholipidgelen (VPG) zur Therapie solider Tumoren; In vitro-Untersuchungen zu Cisplatin-bzw. Vindesin-haltigen VPG, in vitro und in vivo-Untersuchungen zu Vincristin-haltigen VPG. Ph.D. Thesis, Albert-Ludwigs-University of Freiburg, Germany.

Hierholzer, K., Schmidt, R.F., (Eds.), 1991. Pathophysiologie des Menschen. VCH Verlagsgesellschaft, Weinheim, 5.18.

Higuchi, T., 1960. Physical chemical analysis of percutaneous absorption process from creams and ointments. J. Soc. Cosmet. Chem. 11, 85–97.

Holland, J.F., Frei, III, E., Bast, R.C., Jr., Kufe, D.W., Morton, D.L., Weichselbaum, R.R. (Eds.), 1997. Cancer Medicine. Lea & Febiger, Philadelphia, pp. 924–948.

Massing, U., Unger, C., Moog, R., 1998. Verfahren zur Herstellung von liposomalen Wirkstoffformulierungen ("passive loading technique"). International patent application (PCT/EP99/01992).

Mazumder, A., 1981. Effect of liposome-entrapped 5-fluorouracil on Ehrlich ascites tumour bearing mice. Indian J. Biochem. Biophys. 18, 120–123.

Moog, R., 1998. Einschluss von Gemcitabin (dFdC) in vesikuläre Phospholipidgele: in vivo und in vitro-Untersuchungen zur Stabilität, Pharmakokinetik und antitumoralen Wirksamkeit. Ph.D. Thesis, Albert-Ludwigs-University of Freiburg, Germany.

Moog, R., Burger, A.M., Brandl, M., Schuler, J., Schubert, R., Unger, C., Fiebig, H.H., Massing, U., 2002. Change in pharmacokinetic and pharmacodynamic behavior of gemcitabine in human tumor xenografts upon entrapment in vesicular phospholipid gels. Cancer Chemother. Pharmacol. 49, 356–366.

Özer, A.Y., 1992. Stability studies on 5-FU liposomes. Drug Target. Deliv. 1, 151–160.

Peters, G.J., Lankelma, J., Kok, R.M., Noordhuis, P., van Groeningen, C.J., van der Wilt, C.L., Meyer, S., Pinedo, H.M., 1993. Prolonged retention of high concentrations of 5-fluorouracil in human and murine tumors as compared with plasma. Cancer. Chemother. Pharmacol. 31, 269–276.

Rudy, B.C., Senkowski, B.Z., 1973. Fluorouracil. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances, Academic Press, New York, pp. 221–244.

Tardi, C., 1999. Vesikuläre Phospholipidgele: in vitro Charakterisierung, Autoklavierbarkeit, Anwendung als Depotarzneiform. Ph.D. Thesis, Albert-Ludwigs-University of Freiburg, Germany.

Tardi, C., Drechsler, M., Bauer, K.H., Brandl, M., 2001. Steam sterilisation of vesicular phospholipid gels. Int. J. Pharm. 217, 161–172.

Tsukada, S., Ueda, S., Ukada, R., 1984. Preparation of liposome encapsulated antitumor drugs relationship between lipophilicity of and in vitro drug release. Chem. Pharm. Bull. 32, 1929–1935.