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The effect of lipid composition and liposome size on the release properties of liposomes-in-hydrogel

Julia Hurler[a], Simon Žakelj[b], Janez Mravljak[b], Stane Pajk[b,c], Albin Kristl[b], Rolf Schubert[d], Nataša Škalko-Basnet[a,*]

[a] University of Tromsø, Drug Transport and Delivery Research Group, Department of Pharmacy, Universitetsveien 57, N-9037 Tromsø, Norway
[b] University of Ljubljana, Faculty of Pharmacy, Aškerčeva cesta 7, SI-1000 Ljubljana, Slovenia
[c] Institut Jožef Stefan, Laboratory of Biophysics–EPR center, Jamova 39, SI-1000 Ljubljana, Slovenia
[d] Albert Ludwig University, Department of Pharmaceutical Technology and Biopharmacy, Hermann-Herder-Straße 9, D-79104 Freiburg, Germany

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ABSTRACT

To study the release of liposome-associated drugs into hydrogels, we designed and synthesized two pH-sensitive rhodamine derivatives to use as model compounds of different lipophilicities. The dyes were fluorescent when in the free form released from liposomes into the chitosan hydrogel, but not when incorporated within liposomes. The effect of liposomal composition, surface charge and vesicle size on the release of those incorporated dyes was evaluated. The lipophilicity of the rhodamine derivatives affected both the amount and rate of release. While liposome size had only a minor effect on the release of dyes into the hydrogel, the surface charge affected the release to a greater extent. By optimizing the characteristics of liposomes we could develop a liposomes-in-hydrogel system for application in wound therapy. We further characterized liposomes-in-hydrogel for their rheological properties, textures and moisture handling, as well as their potential to achieve a controlled release of the dye. The polymer-dependent changes in the hydrogel properties were observed upon addition of liposomes. The charged liposomes exhibited stronger effects on the textures of the chitosan hydrogels than the neutral ones. In respect to the ability of the system to handle wound exudates, chitosan-based hydrogels were found to be superior to Carbopol-based hydrogels.

1. Introduction

A major aim in the development of modern hydrogel formulations such as those currently used in wound dressings, is to achieve the effective and accurate delivery of the required therapeutic agents included in the formulation over a prolonged period of time (Boateng et al., 2008). Among hydrogels, chitosan hydrogels are one of the most studied systems, particularly with respect to their bioadhesiveness. Chitosan has frequently been studied as a possible wound dressing and as a delivery system for therapeutic agents. This is primarily due to its confirmed biocompatible, biodegradable, non-toxic and bacteriostatic properties, as well as its ability to promote wound healing (Denis et al., 2012). While a lot of research on the potential use of chitosan as a wound dressing has focused on plain chitosan hydrogels, chitosan-based hydrogels (Alsarra, 2009; Bhattarai et al., 2010; Ribeiro et al., 2009), chitosan films (Aoyagi et al., 2007; Noel et al., 2008) and other chitosan-based formulations (Salam et al., 2010), relatively little has been published about liposomal chitosan hydrogels.

The rationale behind using liposomes-in-hydrogel as a delivery system is to assure sustained drug release during their prolonged presence at the administration site (Ruel-Gariepy et al., 2002). The release of drugs from drugs-in-liposomes-in-hydrogel systems is affected by different factors related to the physicochemical properties of the drug. The release of amphiphilic/lipophilic drugs, which are assumed to have the ability to penetrate the liposomal membrane, will be determined by the lipid concentration of liposomes added into the gel (Mourtas et al., 2008b).

In the current study we aimed to gain a better insight into the interactions between drug molecules, liposomes and hydrogels. However, the complexity of the liposomes-in-hydrogel delivery

Abbreviations: ns, non-sonicated; PC, phosphatidylcholine; PC ns, non-sonicated phosphatidylcholine liposomes; PC s, sonicated phosphatidylcholine liposomes; PC/PG, phosphatidylcholine/phosphatidylglycerol; PC/PG ns, non-sonicated phosphatidylcholine/phosphatidylglycerol liposomes; PC/PG s, sonicated phosphatidylcholine/phosphatidylglycerol liposomes; PC/SA, phosphatidylcholine/octadecylamine; PC/SA ns, non-sonicated phosphatidylcholine/octadecylamine; PC/SA s, sonicated phosphatidylcholine/octadecylamine; PG, phosphatidylglycerol; PI, polydispersity index; s, sonicated; SA, octadecylamine (=stearylamine).

* Corresponding author. Tel.: +47 776 46640; fax: +47 776 46151.
E-mail address: natasa.skalko-basnet@uit.no (N. Škalko-Basnet).

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system limits a real-time analytical evaluation of drug release from liposomes, which function as a drug reservoir within the hydrogel, delivering the drug to the administration site. For this purpose, pH-sensitive rhodamine compounds of two different lipophilicities were designed and synthesized to follow their release from liposomes into the hydrogel. The dyes were incorporated in liposomes which varied in lipid composition, surface charge and size.

The use of hydrogels as vehicles provides the required rheological properties required for the incorporated liposomes (Cohen et al., 2012; Mourtas et al., 2007, 2008b; Paavola et al., 2000; Pavelic et al., 2001). In addition, the high viscosity of hydrogels acts as a protective mechanism which can stabilize liposomes, as has been previously shown by Mourtas et al. (Mourtas et al., 2008b).

An additional important characteristic that makes hydrogels interesting for wound therapy is their bioadhesiveness. The rheological and bioadhesive properties of hydrogel formulations determine their retention time at the administration site and can therefore influence the therapeutic outcome of the treatment. Previously, we have shown the superior bioadhesiveness of chitosan-based liposomal hydrogels as compared to Carbopol-based hydrogels (Hurler and Škalko-Basnet, 2012). However, in the case of wound treatment the bioadhesiveness can be affected by the wound's exudate. Some wounds, such as burns, produce a lot of exudate, which can lead to maceration of the wound bed, whereas other wounds are dry and need additional moisture from the wound dressing for their proper healing (Fulton et al., 2012). Therefore, in this study we also tested the fluid handling properties of both chitosan- and Carbopol-based hydrogels.

2. Materials and methods

The rhodamine derivatives used in this study, namely MP-4 and MTJ-12 (log *p* 4.17 and log *p* 2.32, respectively, as calculated by ChemBioDraw 12.0, CambridgeSoft) were synthesized at the Faculty of Pharmacy, University of Ljubljana, Slovenia (manuscript in preparation). Lipoid S100 (PC, soya phosphatidylcholine >94%) and Lipoid E PG-Na (PG, egg phosphatidylglycerol sodium) were a generous gift from Lipoid GmbH (Ludwigshafen, Germany). Octadecylamine (SA) and high Mw chitosan (Brookfield viscosity 800.000 cps, DD of 77) were a product of Sigma–Aldrich Chemistry (St. Luis, USA). Carbopol® Ultrez 10 was obtained from Noveon (Cleveland, USA). Triethylamine was purchased from Merck Schuchardt (Hohenbrunn, Germany) and glycerol was obtained from Merck KGaA (Darmstadt, Germany). All other chemicals used in experiments were of analytical grade.

2.1. Rhodamine derivatives

All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III NMR instrument operating at 400 MHz and 100 MHz (^{13}C). IR spectra were recorded on a PerkinElmer FTIR 1600 spectrometer. Mass spectra were obtained with a Q-Tof Premier mass spectrometer (Centre for Mass Spectrometry, Institute Jožef Stefan, Ljubljana, Slovenia).

3′,6′-bis(ethylamino)-2-(3-hydroxypropyl)-2′,7′-dimethylspiro[isoindoline-1,9′-xanthen]-3-one **MP-4**.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.15–1.19 (m, 2H, N—CH_2–$C\underline{H}_2$—CH_2—OH), 1.22 (*t*, 6H, *J* = 7.25 Hz, 2 × —CH_2—$C\underline{H}_3$), 1.87 (*s*, 6H, 2 × Ar—CH_3), 3.02 (*t*, 2H, *J* = 7.51 Hz, N—$C\underline{H}_2$—CH_2—CH_2—OH), 3.10–3.16 (*m*, 6H, N—CH_2—CH_2—$C\underline{H}_2$—OH, 2 × —$C\underline{H}_2$—CH_3), 4.33 (bs, 1H, —OH), 5.07 (*t*, 2H, *J* = 5.32 Hz, 2 × —NH—), 6.08 (*s*, 2H, $H^{4'}$—Ar, $H^{5'}$—Ar), 6.27 (*s*, 2H, $H^{1'}$—Ar, $H^{8'}$—Ar), 6.96–6.98 (*m*, 1H, H^{7}—Ar), 7.48–7.50 (*m*, 2H, H^{5}—Ar, H^{6}—Ar), 7.77–7.79 (*m*, 1H, H^{4}—Ar) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.15, 17.02, 31.02, 37.27, 37.47, 54,91, 64.28, 95.61, 104.66, 118.16, 122,19, 123,51, 127.51, 128.15, 130.49, 132.52, 147.58, 150.96, 153.64, 166.93 ppm. IR (KBr) 3425, 3337, 2961, 2858, 1682, 1636, 1620, 1517, 1470, 1421, 1326, 1271, 1219, 1159, 1144, 1042, 1014, 868, 814, 782, 746 cm^{-1}. MS (ESI) *m*/*z* (rel intensity) 472 (MH^+, 100); HRMS (ESI): Calcd for $C_{29}H_{34}N_3O_3$ $[M+H]^+$ 472.2600, found 472.2597.

3′,6′-bis(ethylamino)-2′,7′-dimethyl-2-(2-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethyl)spiro[isoindoline-1,9′-xanthen]-3-one **MTJ-12**.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.95 (*t*, 3H, *J* = 6.90 Hz, —CH_2—$C\underline{H}_3$), 1.22 (*t*, 3H, *J* = 7.16 Hz, —CH_2—$C\underline{H}_3$), 1.88 (*s*, 3H, Ar—CH_3), 2.05 (*s*, 3H, Ar—CH_3), 2.91–3.28 (*m*, 12H, N—$C\underline{H}_2$—$C\underline{H}_2$—O—, 2 × —$C\underline{H}_2$—CH_3, H^{2}—G, H^{3}—G, H^{4}—G, H^{5}—G), 3.50–3.53 (*m*, 1H, H^{6a}—G), 3.74–3.79 (*m*, 1H, H^{6b}—G), 4.06–4.15 (*m*, 1H, —OH), 4.54–4.58 (*m*, 2H, 2 × —OH), 4.65 (bs, 1H, —OH), 4.89 (d, 1H, *J* = 4.9 Hz, H^{1}—G), 4.93 (*t*, 1H, *J* = 4.0 Hz, —NH—), 5.18 (*t*, 1H, *J* = 5.14 Hz, —NH—), 6.12 (*s*, 1H, $H^{4'}$—Ar), 6.29 (*s*, 2H, $H^{1'}$—Ar, $H^{8'}$—Ar), 7.01–7.04 (*m*, 1H, H^{7}—Ar), 7.31 (d, 1H, *J* = 5.37 Hz, $H^{5'}$—Ar), 7.50–7.52 (*m*, 2H, H^{5}—Ar, H^{6}—Ar), 7.81–7.79 (*m*, 1H, H^{4}—Ar) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.18, 14.27, 17.05, 17.78, 37.53, 42.05, 48.64, 58.06, 61.64, 64.01, 70.40, 70.56, 78.13, 78.35, 92.97, 93.04, 95.59, 104.09, 114.01, 114.17, 118.64, 122.50, 123.73, 127.53, 128.29, 128.51, 130.23, 132.90, 147.93, 148.82, 148.85, 149.40, 149.47, 150.81, 153.23, 167.05. ppm. IR (KBr) 3422, 2926, 1670, 1522, 1495, 1400, 1270, 1201, 1076, 1016, 888, 747 cm^{-1}. MS (ESI) *m*/*z* (relative intensity) 620 (MH^+, 100); HRMS (ESI): Calculated for $C_{34}H_{42}N_3O_8$ $[M+H]^+$ 620.2972, found 620.2971.

2.2. Preparation and characterization of liposomes

Liposomes were prepared by the dry film method. Three different lipid compositions were used for the preparation: namely PC, PC/PG (1/9, molar ratio), and PC/SA (9/3, molar ratio) (Pavelic et al., 2005). The empty liposomes were used for the rheological and textural studies. In brief, the lipid components (26 mmol/l) were dissolved in methanol and the solvent later removed by evaporation on a rotary vacuum evaporator (Büchi R-124, Büchi Labortechnik, Flawil, Switzerland). The lipid film was rehydrated in 10 ml of distilled water (pH 6.7) and hand-shaken for 10 min. The liposome suspension was kept in a refrigerator overnight before the size reduction and further characterization.

Liposomes containing dyes were prepared in the same manner. Namely, the lipid components were dissolved in methanol and rhodamine dye was added in the organic solution (2 μmol/l). The rhodamine dyes, MP-4 and MTJ-12 (Fig. 1) served as the model fluorescent compounds and were especially synthesized to have the targeted lipophilicity. The dyes were designed to be fluorescent only at a pH value of 4 while being non-fluorescent at pH values higher than 6. The solvent was removed by evaporation and the lipid/compound film rehydrated by 10 ml of phosphate buffer (pH 7.4) and hand-shaken for 10 min prior to storage at 4 °C overnight. To remove unentrapped rhodamine dye the liposomal suspension was ultracentrifuged (80000 *g*, 30 min, Sorvall® WX 100, Thermo Scientific, Waltham, Massachusetts, USA) and the pellet resuspended in 10 ml of distilled water (pH 6.7).

Liposomes of various sizes were prepared by the probe sonication; the liposomal suspensions were cooled in an ice bath and sonicated three times at continuous cycle for 20 s at 40% amplitude by a Cole Parmer Ultrasonic Processor 500 W (Cole Parmer Instruments, Vernon Hills, Illinois, USA).

All liposomal suspensions were characterized for size by dynamic light scattering and zeta potential with a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK).