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ORIGINAL ARTICLE

Preparation and evaluation of liposomes encapsulating synthetic MMP inhibitor (Ro 28-2653)—cyclodextrin complexes

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Abstract In this study, preparation and evaluation of liposomes, intended for intravenous administration, encapsulating synthetic MMP inhibitor (Ro 28-2653) – cyclodextrin complexes were realized. An increase in Ro solubility, via formation of binary (Ro/HPβCD) or ternary (Ro/HPβCD/L-lysine) complexes, permitted a similar increase in encapsulation efficiency of liposomes (Table 1). Moreover, Ro release kinetics depend on the encapsulation efficiency.

Keywords Matrix metalloproteinase inhibitor · Ro 28-2653 · Liposome · Cyclodextrin · Release kinetics

Introduction

Ro 28-2653 (Ro), a synthetic inhibitor of matrix metalloproteinases (MMPs), presents a high selectivity towards three MMPs seeming to play a major role in tumor development and aggressiveness: MMP2, MMP9 and MT1-MMP [1–3]. Targeting of this agent to its activity site is a way to increase efficacy and to decrease adverse side effects. Liposomes are safe and attractive candidates for site-specific drug delivery. However, Ro 28-2653 is a drug that exhibits poor water-solubility and the loading of liposomes with a water-insoluble drug would be limited to the lipid bilayers.

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The aim of this work was to prepare and to evaluate liposomes, intended for intravenous administration, containing Ro/hydroxypropyl-β-cyclodextrin (HPβCD) binary complexes or Ro/HPβCD/L-lysine ternary complexes in the aqueous compartment, with the purpose of increasing encapsulation efficiency.

Experimental

Solutions used for Ro incorporation in liposomes

Ro aqueous solutions containing HPβCD (10 or 40 mM) with or without L-lysine (10 or 50 mM) dissolved in an isotonic pH 7.4 solution of HEPES 10 mM were used for entrapment of inclusion complexes into liposomes. A Ro methanolic solution was used for entrapment of Ro into liposomal bilayers.

Liposomes preparation

Small unilamellar vesicles (phosphatidylcholine:cholesterol:mPEG(2000)distearoyl-phosphatidylethanolamine 62:33:5%mol) were prepared by the thin layer evaporation technique and hydration of lipid films. Suspensions were extruded through polycarbonate membranes of successive 0.4 μm and 0.2 μm pore diameters. Free Ro was separated from liposome-encapsulated Ro by three ultracentrifugations at 35,000 rpm for 90 min at 4 °C.

Liposomes characterisation

Liposome size was measured by photon correlation spectroscopy (HPPS, Malvern Instruments).

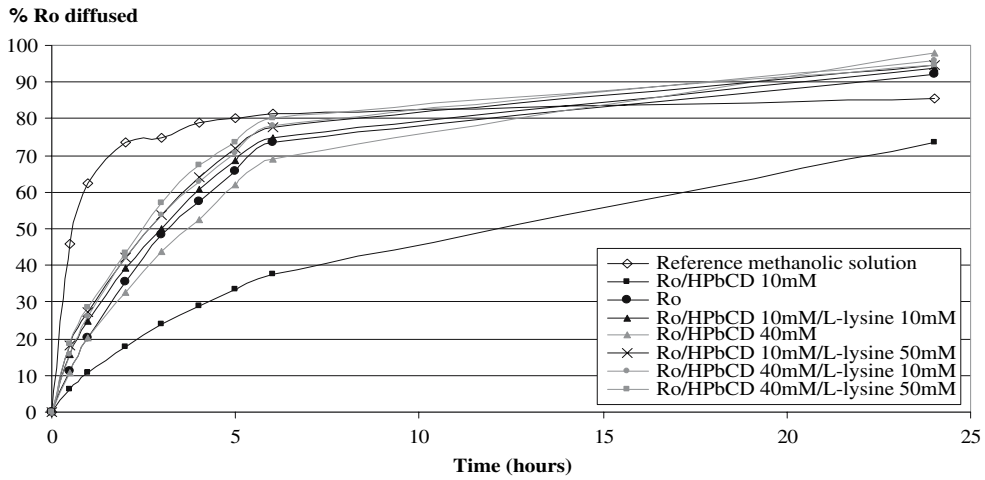

Fig. 1 Comparison of Ro release kinetics from reference methanolic solution containing free Ro (◇) and from liposomes containing Ro (●) Ro binary complexes (■ ▲) or Ro ternary complexes (▲×●■)

Table 1 Ro concentration of different solutions used for Ro incorporation in liposomes, size and encapsulation efficiency (mean ± SD) of liposomes containing free Ro or Ro binary or ternary complexes

Liposomes containing	Ro concentration of used solutions (mM)	Size (nm)	Encapsulation efficiency (%)
Ro/HPβCD 10 mM	0.36	202.9 ± 1.4	1.13 ± 0.06
Ro	0.60	218.7 ± 1.5	2.81 ± 0.17
Ro/HPβCD 10 mM/L-lysine 10 mM	2.33	216.7 ± 6.5	6.98 ± 0.91
Ro/HPβCD 40 mM	2.91	212.3 ± 2.9	4.82 ± 0.49
Ro/HPβCD 10 mM/L-lysine 50 mM	4.70	218.3 ± 6.4	7.92 ± 0.76
Ro/HPβCD 40 mM/L-lysine 10 mM	7.23	233.9 ± 11.6	9.70 ± 3.34
Ro/HPβCD 40 mM/L-lysine 50 mM	16.16	258.0 ± 7.35	15.34 ± 1.33

Phosphatidylcholine concentrations (C_{PC}) were determined by enzymatic colorimetric method (Phospholipids B, Wako) and Ro (C_{Ro}) was assayed by a validated HPLC method [4] in purified liposomes. Encapsulation efficiency was evaluated using the following relation: encapsulation efficiency = (C_{Ro}/C_{PC}) × 100.

In vitro release kinetics of Ro from the different liposomal formulations and from a reference methanolic solution were compared using Franz diffusion cells. The receiver chamber was filled with an isotonic pH 7.4 solution of HPβCD 10 mM and HEPES 10 mM (SINK conditions). A 350 µl volume of preparation, previously diluted in order to obtain a final Ro concentration of 300 µg/ml, was placed into the donor chamber. A polycarbonate membrane of 0.1 µm pore size was clamped between both compartments.

Results and discussion

An increase in Ro solubility, via formation of binary (Ro/HPβCD) or ternary (Ro/HPβCD/L-lysine) complexes, permitted a similar increase in encapsulation efficiency of liposomes (Table 1).

Ro release kinetics depend on the encapsulation efficiency: the rank order observed for Ro release after 6 h corresponds to the rank order observed for encapsulation efficiency, except for liposomes containing Ro in lipid bilayers (their release was faster compared to liposomes containing inclusion complexes in aqueous compartment) (Fig. 1).

A greater size was also observed but not explained for liposomes containing Ro/HPβCD 40 mM in presence of L-lysine.

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