Modified Gemcitabine-loaded lipid nanocapsules: when a drug participates to the structure of a nanomedicine

E. Moysan, G. Bastiat, A. Cieslak, N. Lautram, J. Béjaud, and J.-P. Benoit

Micro et nanomédecines biomimétiques UMR_S 1066, IBS-CHU, Angers, 49933 Cedex 9, France
guillaume.bastiat@univ-angers.fr

ABSTRACT SUMMARY

Lauryl-modified gemcitabine (GemC12) was encapsulated in lipid nanocapsules (LNC) with a high entrapment efficiency and a hydrogel was spontaneously formed, depending on LNC concentration and drug loading. GemC12 was localized in the surface layer of LNCs and the gemcitabine moieties of GemC12, exposed to water medium, formed inter-LNC H-bonds, and therefore an association between LNCs like a pearl necklace occurs. In diluted phases, GemC12-loaded LNCs presented a cytotoxic activity in regards of various cancer cell lines, higher than the native drug.

INTRODUCTION

Gemcitabine (Gemzar®) is a synthetic nucleoside analogue of cytidine. It showed an antitumor activity as a monotherapy against pancreatic cancer and in combination with other chemotherapeutic agents for various cancers.[1] The use of this agent in clinical practice is limited by (i) a dose-dependent toxicity, (ii) a short half-life due to its rapid inactivation by deamination and (iii) resistance mechanism developments for cancer cells.[2,3] Modified gemcitabine with aliphatic chains was previously developed and encapsulated in liposomes.[4] The protection against metabolization of the drug was observed. Besides, the efficacy of this drug was improved compared to the non-modified gemcitabine.[4]

The aim of this work was to develop an innovative formulation of modified gemcitabine-loaded lipid nanocapsules (LNC), in the context of a pharmaceutical nanoplatform widely described by UMR_S 1066 (Angers University, France).[5] To assess the localization of modified gemcitabine in LNCs, drop tensiometer was used. It gave insights on the hydrogel obtained at high LNC concentrations. The self-assembly of LNCs was determined using rheology. Finally, the efficacy of modified gemcitabine-loaded LNCs was tested on pancreatic and lung cancer cell lines.

EXPERIMENTAL METHODS

Gemcitabine was modified with lauryl chain *via* amide function as already described,[4] to finally obtain pure modified gemcitabine (GemC12) characterized by ^{1}H-NMR.

Nanocapsules were formulated based on the principle of the phase inversion process to obtain LNCs structured as a triglyceride core surrounded by lecithins and a nonionic surfactant as already described.[5] GemC12 or lauric acid was added at the beginning of the formulation process. Hydrodynamic diameter and polydispersity index (PdI) were measured using dynamic light scattering (Zetasizer® Nano Series DTS 1060, Malvern Instruments S.A., UK). The drug loading and entrapment efficiency were determined by HPLC method (4,6x150mm C_{18}-column, solvent: methanol, flow: 0.8 mL/min), after LNC disruption with methanol.

Elastic and viscous modulus of LNC formulations were determined by rheology (Kinexus®, Malvern Instruments S.A., UK) (strain: 0.01% and frequency: 1 Hz). Rheological properties were examined with various LNC concentrations and drug loadings. Besides, the nature of the association to obtain the hydrogel form of GemC12-loaded LNCs was studied by rheology with (i) lauric acid-loaded LNCs and (ii) with the addition of urea, sodium choride, ethanol to determine the type of interaction in GemC12-loaded LNCs.

A triglyceride oil drop was formed in aqueous medium using a drop tensiometer (Tracker, IT Concept, France) and surface tension at the interface oil-water was measured with various GemC12 concentrations.

The cytotoxicity of diluted GemC12-loaded LNCs was determined on various cell lines: H460 drug-resistant lung cancer, A549 drug-sensitive lung cancer, Mia-PaCa 2 drug-resistant pancreatic cancer and BxPc-3 drug-sensitive pancreatic cancer, using MTS test. Non-loaded LNCs, Gemzar® and pure GemC12 (in water-ethanol-Tween 80 mixture) were used as control.

RESULTS AND DISCUSSION

Gemcitabine was modified with a lauryl chain (GemC12) to increase the hydrophobicity of gemcitabine. With this modified drug, a direct encapsulation can be envisaged in lipid nanoparticle. LNCs were formulated and GemC12 was added at the beginning of the formulation process. A phase inversion zone was observed as already described in the process, and after the rapid final cold dilution, a hydrogel was obtained spontaneously. As confirmed by rheology, the gel effect was dependent on the GemC12 drug loading and LNC concentration (Figure 1). At drug loading higher than 2.5% w/w_{oil}, LNC hydrogel was observed. Besides, with water dilution, G' and G'' decrease whatever the drug loading above 2.5% w/w_{oil}.

This physical hydrogel was diluted with water and nanocapsules were obtained with a hydrodynamic diameter of 50 ± 4 nm and PdI < 0.1. This size corresponded to that obtained when LNCs were formulated without drug. Besides, without the drug, no hydrogel were obtained as observed in figure 1. The hydrogel was formed by the association of LNCs, due to GemC12 interactions. Whatever the physical form of the formulation: gel (concentrated LNCs) or suspension

(diluted LNCs), drug loading, size and PdI stabilities were measured over time (for 5 months) and no significant change was observed.

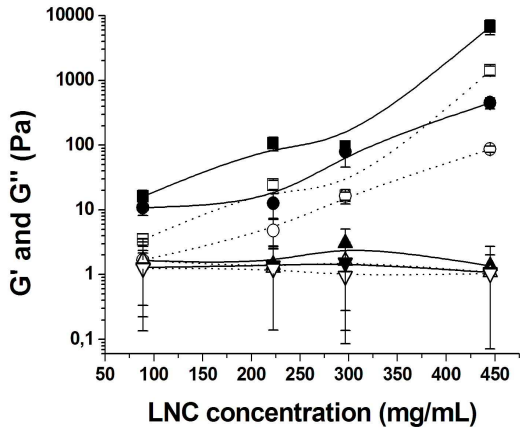

Figure 1. Elastic (G', close symbol) and viscous (G'', empty symbol) *vs.* LNC concentration with various GemC12 drug loadings: 10% (■/□), 5% (●/○), 2.5% (▲/△), and 0% *w/w*$_{oil}$ (▼/▽) (n=3, mean ± SD).

Localization and role of GemC12 were determined. Increasing GemC12 concentration from 0 to 0.6% *w/w*$_{oil}$, surface tension of the oil-water interface decreased from 35 to 15 mN/m, respectively, meaning that GemC12 was located at the interface, around the oily core of LNCs like the surfactant used for the formulation. Therefore this drug actively participated to the formation of the LNCs, explaining the high entrapment efficiency obtained.

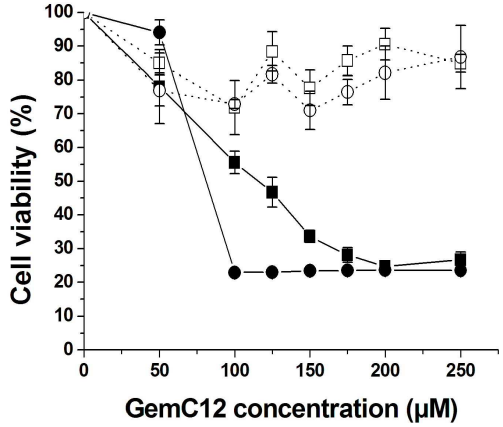

Figure 2. Mia-PaCa 2 cell viability *vs.* Gem-C12 concentration for GemC12 (5% *w/w*$_{oil}$)-loaded LNCs (■), blank LNCs (□), GemC12 (in water-ethanol-Tween 80 mixture) (●) and Gemzar® formulations (○) (n=3, mean ± SD). Incubation time was 48 h at 37°C. Blank LNCs and GemC12(5% *w/w*$_{oil}$)-loaded LNCs were compared at the same particle concentration, reported in GemC12 concentration.

On the contrary, lauric acid-loaded LNCs did not display spontaneous hydrogel formation. Gem moiety of GemC12, oriented on the water side, was responsible to the hydrogel formation with an assumed association between LNCs like a pearl necklace. Rheological experiments were performed on GemC12-loaded LNC hydrogel formulations, with the addition of sodium chloride and ethanol. Gel property was still present showing that the interactions between GemC12 at LNC surface were not due to electrostatic or van der Waals forces. On the other hand, with the addition of urea, gel property was lost meaning that the association inside the hydrogel was due to inter-LNCs H-bonds. This was further confirmed by heating the hydrogel that led to the loss of gel properties.

Finally, once the hydrogel was diluted, the cytotoxic activity of GemC12-loaded LNCs was demonstrated on various lung (H460 and A549) and pancreatic (BxPc-3 and Mia-PaCa 2) cancer cell lines, while no cytotoxic activity was found with non-loaded LNCs at the same dilution (Figure 2). The cytotoxic effect was comparable to pure GemC12 (in water-ethanol-Tween 80 mixture incubation). Besides, this effect was improved compared to the native drug, gemcitabine, as already reported for other system with modified gemcitabine.[4]

CONCLUSION

An innovative nanomedicine was developed with the effective encapsulation of modified gemcitabine in lipid nanocapsules. The original pharmaceutical technology form depending on LNC concentration, *i.e.* hydrogel or suspension, will be interesting to consider for subcutaneous or systemic administrations, respectively. Once diluted, the cytotoxic activity of loaded-LNCs was confirmed, without LNC surface modification.

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