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Lauroyl-gemcitabine loaded lipid nanocapsules for the treatment of metastases in mediastinal lymph nodes.

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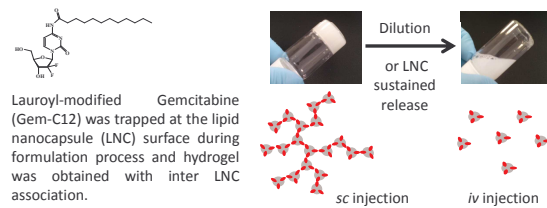
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INTRODUCTION

Lung cancers remain the leading cause of cancer-related mortality around the world (SIEGEL et al, *CA Cancer J. Clin.*, 2013). Once diagnosed, non-small cell lung cancers (NSCLC) 5-year survival rate reaches globally 15% with the different therapeutic modalities such as surgery, radiotherapy and chemotherapy. For most patients, NSCLC had spread in mediastinal and supraclavicular lymph nodes (N2 disease, stage III) or in the contralateral lung, pleural cavity or beyond lung (M1 disease, stage IV) (FUNG et al, *J. Carcinog.*, 2012). In aim to protect the drug until the site of action, target the lymph nodes invaded by metastases and decrease the related systemic toxicities, nanomedicine could be a very great tool (SCHROEDER et al, *Nat. Rev. Cancer*, 2012).

Lipid nanocapsules (LNC) loaded with a lauroyl-modified gemcitabine (Gem-C12) have been developed and demonstrated the ability to form a hydrogel by nanoparticle assembly (MOYSAN et al, *Soft Matter*, 2013). These nanocarriers could be delivered as a gel by subcutaneous (sc) route or as a suspension (after dilution) for intravenous (iv) administration. The purpose of this study is to reach passively the lymph nodes *in vivo*, and to evaluate the antitumor efficacy of this new system in a human NSCLC metastatic model, which mimics the spreading of metastases in mediastinum from the primary tumor implanted in the lung of mice. The tolerance (myelosuppression and hepatotoxicity) of this new treatment delivered either as a gel by sc route or as a suspension by iv route was also investigated.

LAUROYL-MODIFIED GEMCITABINE-LOADED LIPID NANOCAPSULES

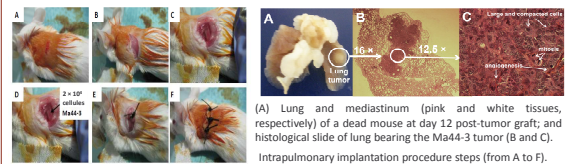


	Z-Ave (nm)	PdI	ζ-Pot* (mV)
Non-loaded LNC (iv)	68 ± 3	0.08 ± 0.02	-8 ± 3
Non-loaded LNC (sc)	67 ± 2	0.07 ± 0.01	-10 ± 5
LNC Gem-C12 (sc)	53 ± 2	0.07 ± 0.01	-7 ± 4
LNC Gem-C12 (iv)	53 ± 1	0.04 ± 0.01	-7 ± 3
DiD-Gem-C12 loaded LNC (sc)	56 ± 2	0.04 ± 0.02	-5 ± 2
DiD-non-loaded LNC (iv)	55 ± 2	0.06 ± 0.02	-7 ± 4

Physicochemical characteristics of loaded and non-loaded LNC for iv and sc injections (n=3; mean ± SD).

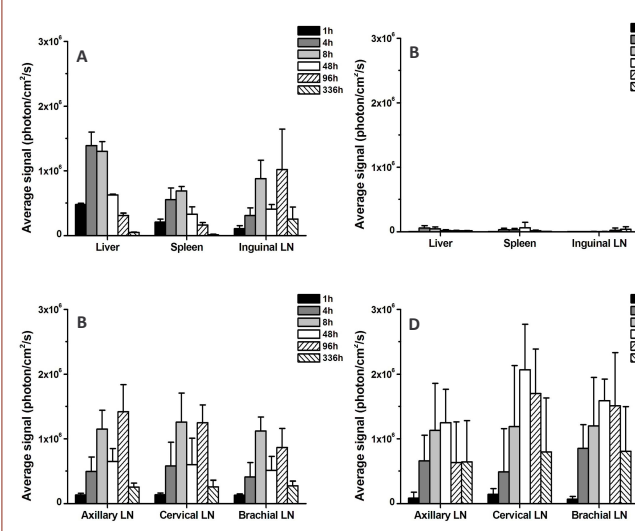
From MOYSAN et al, *Soft Matter*, 2014

PATIENT-LIKE LYMPHOGENOUS METASTATIC PRECLINICAL MODEL



SCID-CB17 mice died from the mediastinal metastases (10-11th day post-tumor graft) and not from small primary lung tumor.

IN VIVO BIODISTRIBUTION IN HEALTHY AND TUMOR-BEARING MICE

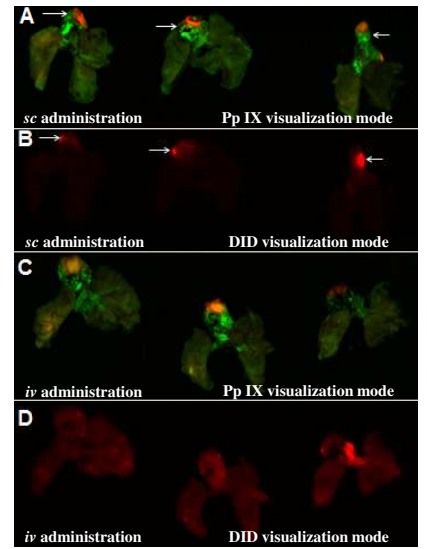


In vivo biodistribution of DiD-LNC in healthy nude mice, after intravenous injection (tail vein) of DiD-LNC in suspension (no encapsulated Gem-C12) (A and B) and subcutaneous injection (behind the neck) of DiD-LNC hydrogel (5% Gem-C12/Labrafac w/w) (C and D). Semi-quantification of the fluorescence signals of the removed liver, spleen and inguinal, axillary, cervical and brachial lymph nodes (LN) using fluorescence images (10 ms integration time) at various times post LNC administrations (1, 4, 8, 48, 96 and 336h). (n=5, mean ± SD).

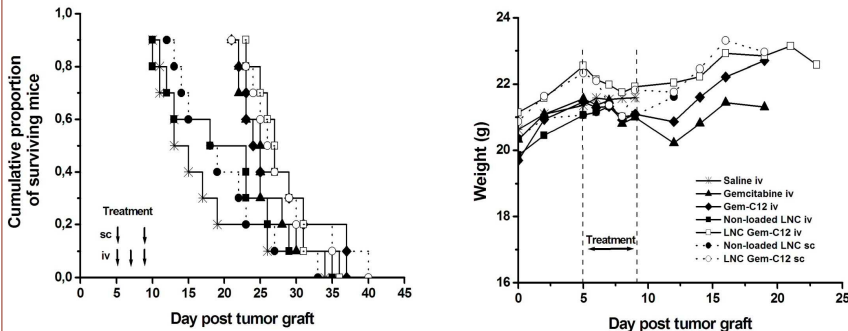
An immediate accumulation in liver and spleen, and in all lymph nodes was observed with DiD-loaded LNC suspension intravenously delivered. DiD-Gem-C12-loaded LNC in gel form subcutaneously delivered accumulated exclusively in the lymph nodes close to the injection site and presented a very low systemic exposition.

With tumor-bearing SCID-CB17 mice, DiD-LNC were visible in the entire lung of the three mice after iv injection. On the contrary, DiD-LNC administered by sc route emitted an intense local accumulation in mediastinal lymph nodes.

Visualization of lungs of three mice at day 5 after tumor implantation, following oral administration of 400 mg/kg of 5-aminolevulinic acid and administration of LNC loaded with DiD subcutaneously delivered (with Gem-C12) using (A) Pp IX or (B) DiD visualization mode; or intravenously delivered (without Gem-C12) using (C) Pp IX or (D) DiD visualization mode using a CRi Maestro system. Arrows pointed the localization of mediastinal lymph nodes in the pictures.



IN VIVO ANTITUMOR EFFICACY AND TOLERANCE OF TREATMENTS



The Kaplan-Meier survival curves and the weight evolution of mice (n=10 per group) grafted with Ma44-3 cells after various gemcitabine treatments (total dose of 40 mg/kg, molar equivalent gemcitabine hydrochloride).

Significant level ($p < 0.05$, log-rank test) was reached with all gemcitabine treatments in comparison to controls (treatment without drug). However, there was no significant difference between the survival rate of groups treated by gemcitabine regardless of the route of administration. The weight evolution of the different mice groups remained similar during the experiment with a short stabilization during the treatment period.

Treatment with non-encapsulated Gemcitabine presented significant decreases ($p < 0.05$, Kruskal-Wallis test) of platelet count and alkaline phosphatase. No difference was measured for plasma biochemical parameters and complete blood counts when Gem-C12 was encapsulated inside LNCs, regardless of the route of administration.

	WBC (giga/L)	RBC (tera/L)	HGB (g/dL)	HCT (%)	PLT (giga/L)
Saline iv	0.2 ± 0.1	9.8 ± 0.3	15.1 ± 0.3	47 ± 1	469 ± 50
Gemcitabine iv	0.2 ± 0.1	9.2 ± 0.3	14.0 ± 0.4	45 ± 1	245 ± 63 *
Gem-C12 iv	0.3 ± 0.2	9.1 ± 0.6	14 ± 1	45 ± 2	275 ± 63
Non-loaded LNC iv	0.6 ± 0.3	9.8 ± 0.2	15.1 ± 0.3	47.7 ± 0.9	465 ± 44
LNC Gem-C12 iv	0.14 ± 0.03	8.9 ± 0.2	13.7 ± 0.3	43.1 ± 0.9	284 ± 24
Non-loaded LNC sc	0.4 ± 0.1	9.4 ± 0.7	14 ± 1	46 ± 3	453 ± 20
LNC Gem-C12 sc	0.26 ± 0.06	9.4 ± 0.5	14.5 ± 0.8	45 ± 2	281 ± 83

	ASAT (UI/L)	ALAT (UI/L)	PH ALK (UI/L)	Creatinine (mg/L)
Saline iv	138 ± 115	24 ± 8	146 ± 8	1.8 ± 0.4
Gemcitabine iv	98 ± 27	27 ± 12	129 ± 17	3 ± 3
Gem-C12 iv	152 ± 95	23 ± 8	97 ± 33 *	2 ± 1
Non-loaded LNC iv	77 ± 20	22 ± 4	139 ± 4	1.7 ± 0.5
LNC Gem-C12 iv	82 ± 21	18 ± 7	143 ± 14	3 ± 2
Non-loaded LNC sc	88 ± 52	24 ± 16	109 ± 5	2 ± 1
LNC Gem-C12 sc	157 ± 52	32 ± 10	121 ± 11	4 ± 3

Biological analysis and complete blood counts in different groups. WBC: complete granulocyte count, RBC: red blood cell count, HGB: hemoglobin rate, HCT: hematocrit, PLT: platelet count, ASAT: aspartate aminotransferase, ALAT: alanine aminotransferase, PH ALK: alkaline phosphatase. (n=5; mean ± SD, *: $p < 0.05$, Kruskal-Wallis test and Dunn's post-hoc test).

CONCLUSION

The lipid nanocapsules loaded with a lipophilic derivative of gemcitabine were able to target the lymph nodes and more specifically the mediastinal lymph nodes, displaying a similar antitumor efficacy as the conventional systemic gemcitabine in the lymphogenous metastatic preclinical model with a treatment schedule of twice a week sc in comparison to three times a week iv for the standard drug. Moreover, LNC loaded with Gem-C12 and delivered iv or sc did not induce myelosuppression unlike the conventional systemic gemcitabine. This latter feature shows clearly the advantage brought by a nanostructured formulation of gemcitabine.

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