Enhanced paclitaxel delivery to tumors using a new lipid nanocapsule-based formulation

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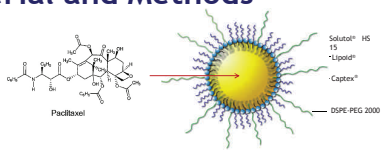
Introduction

TAXOL® (paclitaxel, PTX), one of the first microtubule stabilizing agents, is among the most widely used chemotherapy agents in various cancers, especially ovarian and breast cancer. However, because of its poor water-solubility, PTX must be dissolved in ethanol and Cremophor® EL. Cremophor® EL has been proved to be associated with a number of severe side effects, including hypersensitivity, neurotoxicity and dramatic allergic reactions. These side effects are a major limitation in the use of PTX in the clinic. To avoid these side effects, there is a need to develop alternative formulations of PTX with better aqueous solubility and reduced risk of associated serious adverse effects. In this study, PTX was formulated in Lipid NanoCapsules (LNC), prepared *via* the phase inversion temperature method. The pharmacokinetics/pharmacodynamic (PK/PD) parameters of PTX-LNC were evaluated in a BALB/C nude mice model bearing human ovarian tumor implanted subcutaneously.

Material and Methods

PTX-LNC
Composition : Solutol® HS 15 (stearate de PEG 15), Lipoid® (lécithine de soja), Captex ® (acide caprique et caprilique), DPSE-PEG 2000.



In Vivo studies

- **Test substances**
  - Preparation of PTX loaded LNCs (PTX-LNC)
  - TAXOL® clinical formulation diluted in saline
- **Tumor cell line : human SK-OV-3 ovarian adenocarcinoma**
- **Animals : BALB/C nude mice (CHARLES RIVERS, L'Arbresles, France)**

PD experiment

- **Drug administration:**
  - IV *bolus* of PTX-LNC and TAXOL® at 12 mg eq PTX/kg

Group	Treatment	No animals	Dose (mg PTX/kg/inj)	Adm. Route	Treatment schedule
1	NaCl 0.9%	12		IV	Q1Dx5
2	PTX-LNC	12	12	IV	Q1Dx5
3	TAXOL®	12	12	IV	Q1Dx5

- **Tumor induction and randomisation**
  - SC inoculation of 1x10^7 SK-OV-3 cancer cells into nude mice
  - Randomization of mice based on tumor volumes (150 ± 23 mm^3)
- **Mice monitoring**
  - Daily monitoring of mice survival
  - Managment of the study (dosing, collection, measurements…), raw data, lethality, behaviour and autopsy were recorded and analysed using Vivo Manager software (Biosystems, Dijon) (1) (2)
  - Twice a week monitoring of mice body weight and tumor volumes

PK experiment

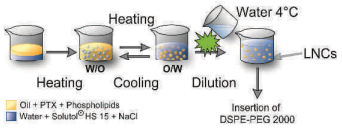
- **Drug administration:**
  - IV *bolus* of PTX-LNC and TAXOL® at 20 mg eq PTX/kg

Group	Treatment	Nb animals	Dose (mg/kg/inj)	Adm. Route	Treatment schedule
1	PTX-LNC	3 *per* timepoints (10 timepoints)	20	IV	Q1Dx1
2	TAXOL®	3 *per* timepoints (10 timepoints)	20	IV	Q1Dx1

- **Tumor induction, randomization and collection**
  - SC inoculation of 1x10^7 SK-OV-3 cancer cells into nude mice
  - Randomization of mice based on tumor volumes (261 ± 42 mm^3)
  - Collection of tumor, plasma, liver, spleen and kidneys
  - Determination of PTX levels in tumor, plasma, liver, spleen and kidneys using HPLC/MS/MS

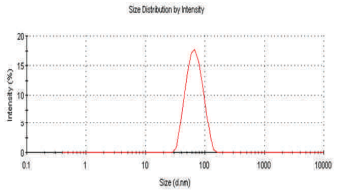
(1). Principe d'éthique de l'expérimentation animale. Directive n 86/609 CEE du 24 Nov. 1986, Décrêt n 87/848 du 19 Oct. 1987, Arrêté d'Application du 19 Avril 1988.
(2). Workman et al. Guidelines for the welfare and use of animals in cancer research. Br J Cancer. 2010;102(11):1555-1577.

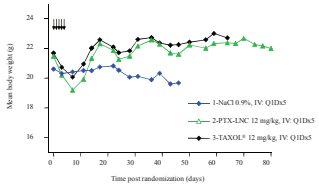
Results



Particles characteristics :

- Mean diameter PTX-LNC : 65 nm (intensity); PdI = 0.08
- ζ Potential : -32.2 mV
- Encapsulation efficiency after DPSE-PEG 2000 insertion : 87 %
- pH = 7.4



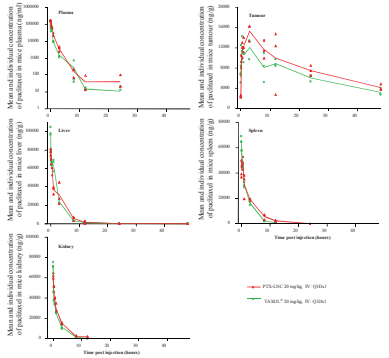


- A significant MBW loss between D0 and D7 was observed for mice treated with PTX-LNC and TAXOL® at 12 mg/kg when compared to mice treated with NaCl 0.9 %
- No significant mean body weight change was observed between PTX-LNC and TAXOL® when administered at the same PTX equivalent dose

Group	No mice at D0	MBWC (D0-D7, %)	SD
1-NaCl 0.9%, IV: Q1Dx5	12	-0.94	3.04
2-PTX-LNC 12 mg/kg, IV: Q1Dx5	12	-10.57***	1.87
3-TAXOL® 12 mg/kg, IV: Q1Dx5	12	-7.34***	4.58

MBWC: Mean Body weight change between D0-D7
MBWC (%): Mean body weight change between D0-D7 compared to the mean body weight at D0
SD: Standard Deviation

Determination of PTX concentration in plasma, tumor, liver, spleen and kidneys originating from BALB/C nude mice bearing subcutaneous SK-OV-3 tumors.
The mice received a single IV injection of PTX-LNC or TAXOL® at 20 mg eq PTX/kg. The points represent the individual PTX concentration of each mouse.



Parameters	PTX-LNC	TAXOL®
	Plasma	**Plasma**
Cmax (ng/ml)	172666	150333
Tmax (hr)	0.083	0.083
$AUC_{0.083 \rightarrow \infty}$	98353	53602
Half-life (Hr)	0.313	0.766
Clairance (ng/kg/hr x ng/g)	203.3	373.1
Vol distribution (ng/kg/ng/ml)	92.0	413.5
Tumor	**Tumor**	**Tumor**
Cmax (ng/ml)	15279	12061
Tmax (hr)	3	3
$AUC_{0.083 \rightarrow \infty}$	392654	316440
Liver	**Liver**	**Liver**
Cmax (ng/ml)	78147	99663
Tmax (hr)	0.083	0.083
$AUC_{0.083 \rightarrow \infty}$	242475	241512
Kidneys	**Kidneys**	**Kidneys**
Cmax (ng/ml)	62538	72634
Tmax (hr)	0.083	0.083
$AUC_{0.083 \rightarrow \infty}$	135582	105609
Spleen	**Spleen**	**Spleen**
Cmax (ng/ml)	47745	65959
Tmax (hr)	0.5	0.083
$AUC_{0.083 \rightarrow \infty}$	167146	138510

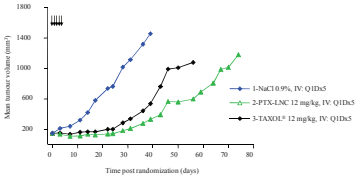
Cmax: Maximal concentration
AUC: Area under the curve

- Similar PTX plasma pharmacokinetic profiles were observed for PTX-LNC and TAXOL® at 20 mg eq PTX/kg in SK-OV-3 tumor bearing mice
- The PTX half-life of elimination in plasma for PTX-LNC was 2.4 fold shorter than for TAXOL®
- The $AUC_{0.083 \rightarrow 8H}$ was 1.24 fold higher for PTX-LNC compared to TAXOL® in tumor

Results

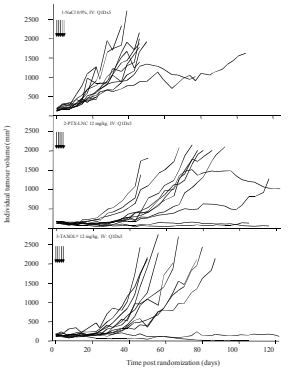
Mean tumor volume curves

Mean tumor volume curves of BALB/C nude mice bearing subcutaneous SK-OV-3 tumors.
The mice received one daily IV injection of PTX-LNC or TAXOL® for 5 consecutive days from D0 to D4 (Q1Dx5). The daily treatments are indicated by arrows. Each point represents the mean of the recorded body weight per group (n=12).



Individual tumor volume curves

A marked and superior inhibition of the tumor growth was observed for mice treated with PTX-LNC when compared to TAXOL® at the same PTX equivalent dose.



Treatment effects on the growth of SK-OV-3 tumors subcutaneously xenografted in BALB/C nude mice.

Group	No mice at D0	Mean TGD (days)	SD
1-NaCl 0.9%, IV: Q1Dx5	12	19.7	6
2-PTX-LNC 12 mg/kg, IV: Q1Dx5	12	54.0***	16
3-TAXOL® 12 mg/kg, IV: Q1Dx5	12	40.2***	8

TGD: Tumour Growth Delay
SD: Standard Deviation

- A significant mean time to reach the tumor volume of 600 mm^3 difference was observed for mice treated with PTX-LNC and TAXOL® when compared to mice treated with NaCl 0.9 %
- A significant 1.3 fold-increase of the mean time to reach the tumor volume of 600 mm^3 was observed for mice treated with PTX-LNC when compared to mice treated with TAXOL® (54 *vs* 40 days, respectively)

Summary table of median tumor volume and T/C % calculated by comparing the median tumor volumes of treated mice with the median tumor volume of vehicle treated mice.

Group	Parameters	D31
1-NaCl 0.9%, IV: Q1Dx5	Median tumour volume (mm³)	1064
	T/C %	100
2-PTX-LNC 12 mg/kg, IV: Q1Dx5	Median tumour volume (mm³)	169
	T/C %	16
3-TAXOL® 12 mg/kg, IV: Q1Dx5	Median tumour volume (mm³)	307
	T/C %	29

T/C%: Ratio of the median tumour volumes of treated group (T) versus vehicle treated group (C)

At D31, the T/C % values were inferior to effective criteria according to NCI standards and were 16 and 29 % for mice treated with PTX-LNC and TAXOL®, respectively.

Treatment effects on tumor volume of SK-OV-3 tumors subcutaneously xenografted in BALB/C nude mice at D28.

Group	No mice at D0	Mean tumour volume at D28 (mm³)	SD
1-NaCl 0.9%, IV: Q1Dx5	12	1018	429
2-PTX-LNC 12 mg/kg, IV: Q1Dx5	12	184***	139
3-TAXOL® 12 mg/kg, IV: Q1Dx5	12	266***	181

SD: Standard Deviation

- A marked and significant decrease of the mean tumor volume at D28 was observed for mice treated with PTX-LNC and TAXOL® when compared to mice treated with NaCl 0.9 %
- A slight, but not significant decrease of the mean tumor volume was observed for the groups of mice treated with PTX-LNC when compared to mice treated with TAXOL® at 12 mg/kg (1.5 fold)

Conclusions

- Despite transient BW losses, PTX-LNC was well tolerated by BALB/C nude mice bearing subcutaneous SK-OV-3 tumors.
- Based upon the evaluation criteria of antitumor activity, a marked inhibition of the tumor growth was observed for mice treated with PTX-LNC superior to that observed with TAXOL® at the same PTX equivalent dose.
- Similar PTX pharmacokinetic profiles were observed when treated with PTX-LNC and TAXOL® at equivalent dose in SK-OV-3 tumor bearing mice. However, the exposure of plasma and tumor to PTX after LNC-PTX injection were increased compared to TAXOL®.