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Note

In vitro and *in vivo* evaluation of camptothecin nanosuspension: A novel formulation with high antitumor efficacy and low toxicity

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ABSTRACT

The purpose of this study was to evaluate the *in vitro* and *in vivo* antitumor efficacy and the dose dependent toxicity of camptothecin nanosuspension (Nano-CPT) comparing with that of topotecan (TPT). A novel supercritical antisolvent (SAS) process-high pressure homogenization technique has been developed to prepare Nano-CPT. The cytotoxicity of Nano-CPT and TPT was investigated against MCF-7, HCT-8, and PC-3 cell lines using MTT assay, antitumor activity *in vivo* were evaluated against HCT-8 xenograft model, and the dose dependent toxicity *in vivo* during the treatment were investigated by body weight changes and relative organ weight variations. The Nano-CPT presents about 6 times *in vitro* cytotoxicity active than TPT against cell lines MCF-7, nearly the same *in vivo* antitumor activity with TPT and lower toxicity. The results confirm that Nano-CPT is a novel potential formulation with high antitumor efficacy and low toxicity.

Camptothecin (CPT), that targets the nuclear enzyme topoisomerase I, is a naturally occurring cytotoxic alkaloid isolated from Chinese plant *Camptotheca acuminata*, which has been known as a potent agent against a wide spectrum of human cancers (Oberlies and Kroll, 2004; Wall et al., 1989). CPT has poor solubility in water and other physiologically acceptable solvents, which limits its clinical application severely. There are two basic approaches to overcome the solubility problem of CPT: (1) to synthesize new water-soluble CPT analogs or (2) to develop novel delivery systems (Zhao et al., 2010). After hundreds of thousands of CPT analogs have been synthesized, only two analogs: topotecan (TPT) and irinotecan (CPT-11) have been approved as anticancer agents by FDA (Cortes et al., 2002; Saijo, 1996). More and more attention has been focused on developing novel delivery systems: liposomes, cyclodextrins, microspheres, microemulsions and other polymers have already been used to prepare new CPT complexes (Cortesi et al., 1997; Kang et al., 2002; Schneider et al., 2008; Moon et al., 2008; Inoue et al., 2003). However, until now there has been no CPT formulation on international market. Development of new drug delivery system (DDS) for CPT is still one of the most important goals in cancer chemotherapy today.

Formulating poorly soluble drugs as nanocrystals can increase their saturation velocity, dissolution velocity and adhesiveness to surfaces/cell membranes, thus increase their bioavailability after oral administration, reduce their large injection volume for intravenous administration, and also decline undesired side effects after intravenous injection when using traditional formulations (Müller et al., 2011). Nanosuspension, defined as a dispersion of drug nanocrystals (<1000 nm) in an outer liquid phase, is a new drug delivery system (Moschwitzer et al., 2004). According to the extremely poor solubility of CPT, we prepared camptothecin nanosuspension (Nano-CPT) using supercritical antisolvent (SAS) process-high pressure homogenization technique, and the *in vitro* and *in vivo* antitumor efficacy and does dependent toxicity of Nano-CPT were evaluated and first time comparing with that of TPT.

SAS process was first used for the micronization of CPT, based on the method used in the early research (Zhao et al., 2010). In brief, four important parameters in the SAS process, *i.e.*, the pressure in the precipitation chamber, the temperature in precipitation chamber, the CPT concentration in dimethyl sulfoxide solution, and the flow rate, were 20 MPa, 35 °C, 1.25 mg mL^{-1}, and 6.6 mL min^{-1}, respectively, and then CPT nanoparticle of 250.6 ± 20.3 nm was obtained.

For high pressure homogenization, 40 mg CPT nanoparticle was poured in 40 ml purified water, and sonicated until the CPT nanoparticle was completely dispersed (Fig. 1A), and the pH of the mixture was adjusted to 6.0 to protect the potent lactone form of CPT. After that the Nano-CPT was prepared using a high pressure homogenizer (NS1001L-PANDA 2K, Niro Soavi, Italy). At first, two cycles at 200 bar and two cycles at 400 bar were conducted as pre-milling, then a high pressure homogenization step was applied on the suspensions at 1300 bar for 20 cycles to obtain the final

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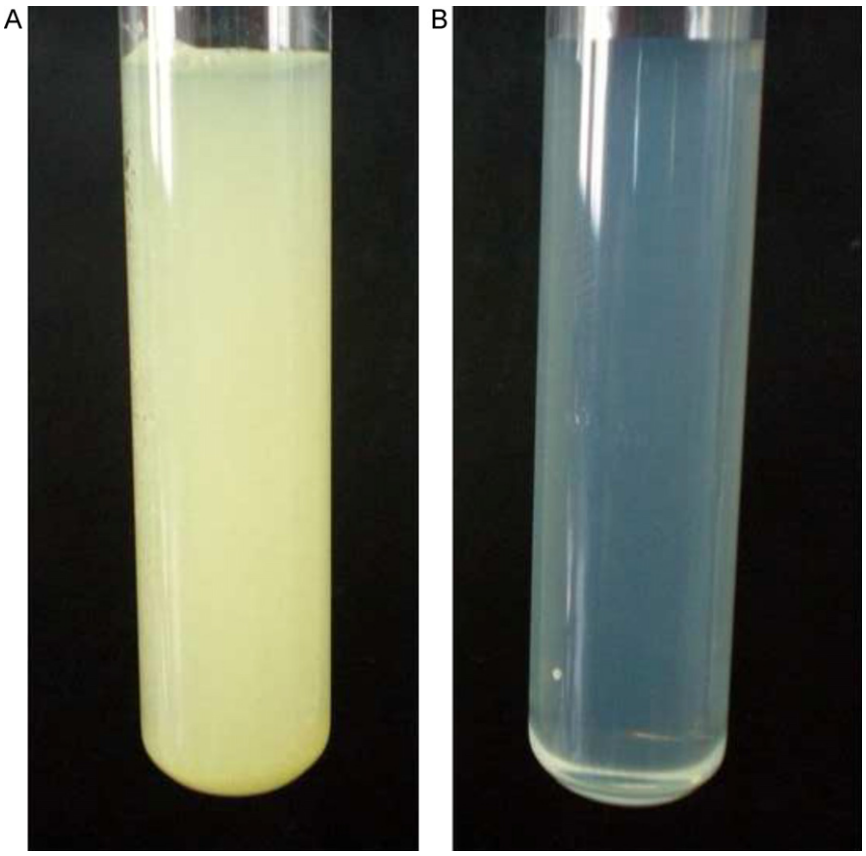

Fig. 1. Morphology of Nano-CPT: (A) after SAS process and (B) after high-pressure homogenization.

Nano-CPT. The Nano-CPT (Fig. 1B) was very transparent, indicating that the CPT particles have been grinded adequately by this technique process (Adkins et al., 2008). Mean diameter of Nano-CPT was 125.1 ± 8.6 nm with a narrow distribution range and measured by LS 230 Laser Diffraction Particle Size Analyzer (Beckman Coulter, USA), Nano-CPT was freeze-drying by using mannitol as a lyoprotectant to maintain particulate nature for a long time.

The cytotoxicity of CPT (dissolved in DMSO, final concentration ≤ 3%), TPT (water solution) and Nano-CPT (water solution) against three different cancer cell lines was evaluated using standard MTT assay (Table 1). The Nano-CPT has a little lower antitumor activity than CPT in each cell line, but more than 3 times, 4 times and 6 times higher activity than that of TPT against human colorectal carcinoma cell line HCT-8, human prostate carcinoma cell line PC-3, and human breast adenocarcinoma cell line MCF-7, respectively, which indicates that the cytotoxicity of Nano-CPT were nearly the same as that of CPT, but more effective than that of TPT.

The *in vivo* antitumor activity of Nano-CPT was evaluated against nude mice bearing HCT-8 xenograft model, following a five days on and two days off treatment schedule (qd × 5/weekly, 1 mg/kg or 2 mg/kg, i.v.). Table 2 lists the tumor inhibition rates of all the tested groups. In all groups, excluding the negative control (injected with 0.9% NaCl), the tumor inhibit rates were ≥90%. The *in vivo* antitumor evaluation indicates that Nano-CPT has an excellent antitumor activity nearly the same as TPT. Fig. 2 shows photograph of excised sarcomas from the tested groups, which provide a direct visual representation of the tumor-suppression effect. It can be apparently seen that in the negative control group, the tumors have an irregular shape with ill-defined borders and scarlet color, indicating that the tumor was receiving sufficient blood supply and its growth was under no suppression condition. In contrast, for the groups treated with TPT and Nano-CPT, the excised tumors have a markedly smaller size with much more clear borders, suggesting the tumor growth was greatly suppressed. Moreover, the tumor color was pale-red as a consequence of an obvious suppression in the tumor vascular system.

Single dose acute toxicity test has been carried out by non-tumor-bearing BALB/c mice (6 or 10 animals per dose group)

Table 1
Cytotoxicity of TPT, CPT and Nano-CPT.

	MCF-7	HCT-8	PC-3
TPT	12.55 ± 0.89	6.824 ± 0.46	2.576 ± 0.23
CPT	0.56 ± 0.16	1.48 ± 0.25	0.48 ± 0.12
Nano-CPT	2.07 ± 0.34	2.09 ± 0.42	0.61 ± 0.15

Compound *in vitro* cytotoxicity (IC_{50}, μM).

Table 2
In vivo antitumor activity of TPT and Nano-CPT.

Compound	Dose (mg/kg)	Lethal[a] toxicity	BWC (%)[b]	TIR[c] (%)
Control	–	0/6	+12	–
TPT	1	0/6	−10	92.04 ± 2.24**
	2	1/6	−18	95.07 ± 0.94**
Nano-CPT	1	0/6	−10	94.27 ± 3.36**
	2	0/6	−13	95.47 ± 3.30**

[a] Number of the dead mice/total number of mice.
[b] Percentage of mice body-weight change (BWC) after drug treatment: BWC% = (mean BW final day/mean BW first day × 100) − 100; +, means body-weight increase; −, means body-weight decrease.
[c] Tumor inhibitory rate.
** $p < 0.01$, *versus* the control group.

Table 3
The relative organ weight of TPT and Nano-CPT.

Compound	Dose (mg/kg)	Relative organ weight[a] (%)				
		Liver	Kidney	Spleen	Heart	Lung
Control	–	6.35 ± 0.53	1.54 ± 0.13	0.80 ± 0.13	0.55 ± 0.06	0.94 ± 0.09
TPT	1	5.01 ± 1.09*	1.31 ± 0.15*	0.37 ± 0.07**	0.58 ± 0.06	0.73 ± 0.09**
	2	3.70 ± 0.26**	1.26 ± 0.30**	0.19 ± 0.04**	0.57 ± 0.12	0.75 ± 0.05**
Nano-CPT	1	5.35 ± 0.91*	1.44 ± 0.09	0.40 ± 0.14**	0.55 ± 0.05	0.88 ± 0.20
	2	4.73 ± 0.92*	1.38 ± 0.08	0.25 ± 0.07**	0.52 ± 0.06	0.83 ± 0.18

[a] Relative organ weight (%) = (mean organ weight/mean body weight) × 100.
* $p < 0.05$, *versus* the control group.
** $p < 0.01$, *versus* the control group.

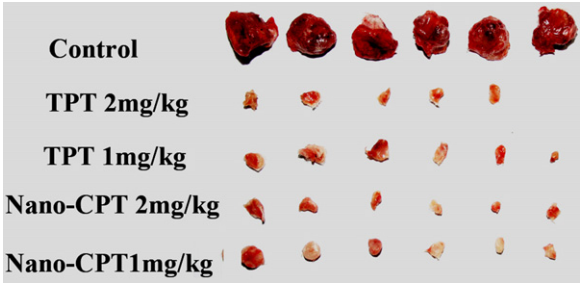

Fig. 2. Photographs of excised tumors from mice treated with negative control, TPT and Nano-CPT.

for the purpose of does finding, CPT or Nano-CPT and TPT at 2, 3, 4.5, 6.7 and 10 mg/kg (DMSO ≤ 3%, i.p.) or 9, 13.4, 20, 30, 45 and 67 mg/kg (i.v.) were investigated. Dose and mortality curves were calculated using the Boltzmann sigmoidal equation (GraphPad software, San Diego, CA). The maximum tolerated does (MTD) was defined as the highest dose level with ≤10% mortality. It has been shown that, the MTD of Nano-CPT was 24 mg/kg, which means the *in vivo* toxicity of Nano-CPT was significantly lower than those of CPT (MTD 3 mg/kg) and TPT (MTD 15 mg/kg).

The body weights and relative organ weights of mice were measured for investigating the dose dependent toxicity during the *in vivo* antitumor evaluation. As shown in Table 2, the body weight gain of mice was significantly suppressed in the treatment group during the treatment period when compared with the negative control group, but the average weight loss of each group were not less than 15%, except the TPT 2 mg/kg group, which means the TPT 2 mg/kg group has the most toxicity to mice body system. The relative organ weights of liver, kidney, spleen and lung were significantly decreased in the TPT groups (Table 3), and in the Nano-CPT group only the relative weights of liver and spleen were decreased significantly, which means Nano-CPT has less toxicity to lung and kidney which are the target toxic organs of TPT. According to the results, Nano-CPT has much less toxicity *in vivo* compared with TPT at the same dose, especially not exhibited significantly toxicity for lung and kidney.

In conclusion, this study suggests that Nano-CPT is a novel formulation with high antitumor efficacy, low toxicity, and has an excellent potential to be developed as a new agent for cancer therapy.

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