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Pharmacokinetics of hydroxycamptothecin nanosuspensions in rats

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The purpose of the present study was to investigate the pharmacokinetics of hydroxycamptothecin nanosuspensions after intravenous administration in rats. Hydroxycamptothecin injection was studied parallelly. The results showed that $AUC_{0\rightarrow\infty}$, MRT, $t_{1/2(\alpha)}$ and $t_{1/2(\beta)}$ of hydroxycamptothecin nanosuspensions was significantly higher, while their total body clearance was lower than those of hydroxycamptothecin injections. The results indicate that hydroxycamptothecin nanosuspensions significantly increase hydroxycamptothecin blood concentrations and retention within the systemic circulation.

Nanosuspensions are submicron colloidal dispersions of pure drug nanocrystals in an outer liquid phase (Moschwitzer et al. 2004) and are particularly used to administer to water insoluble drugs e.g., orally, parenterally, pulmonarily and ocularly (Jacobs and Muller 2002; Keck and Müller 2006; Pignatello et al. 2002). The major potential clinical benefits of a nanosuspension dosage form include (1) high drug loading leading to lower volume of injection, (2) reduction in toxicity by replacing solubilizing agents, like Cremophor, with relatively low quantity of suspension stabilizing surfactants, (3) possibility of altering the pharmacokinetic profiles of the drug leading to higher dosing, more efficacious regimens and less frequent administration, and (4) possibly passive or even active targeting for drug delivery (Wong et al. 2008). Hydroxycamptothecin (HCPT) is a promising anticancer agent along with its analogues. The α-hydroxy-δ-lactone ring of HCPT is crucial for its anti-tumor activity (Hertzberg et al. 1989). At present, HCPT injections are formulated as a sodium salt of the carboxylate which possess only 10% of the cytotoxic activity of lactone form (Hertzberg et al. 1989), which has exhibited several side-effects in clinical trials (Wani et al. 1980; Zhang et al. 2001). Therefore, the therapeutic potential of HCPT has been limited by its low aqueous solubility, *in vitro* and *in vivo* instability (Burke and Mi 1993). Recently, we developed a novel precipitation-combined high-pressure homogenization technique to prepare hydroxycamptothecin nanosuspensions (HCPT-NSs), which proved to have higher cytotoxicity against the cancer cells than HCPT injections (HCPT-I, Zhao et al. 2010). In the present work, we tried to investigate whether nanosuspensions can modify the pharmacokinetics of HCPT.

Table: Pharmacokinetics parameters of HCPT after i.v. administration of HCPT-NSs and HCPT-I to rats with dose 4 mg kg^{-1} (n = 6)

Parameters	HCPT-NSs	HCPT-I
$t_{1/2(\alpha)}$ (h)	0.19 ± 0.03	0.09 ± 0.03
$t_{1/2(\beta)}$ (h)	3.40 ± 0.64	0.57 ± 0.14
MRT (h)	4.67 ± 0.74	0.82 ± 0.20
Vc (L·kg^{-1})	0.93 ± 0.13	1.142 ± 0.260
CL (L·h^{-1}·kg^{-1})	2.76 ± 0.30	7.37 ± 2.17
$AUC_{0\rightarrow\infty}$ (h·ng·mL^{-1})	1467.29 ± 170.27	621.19 ± 180.95

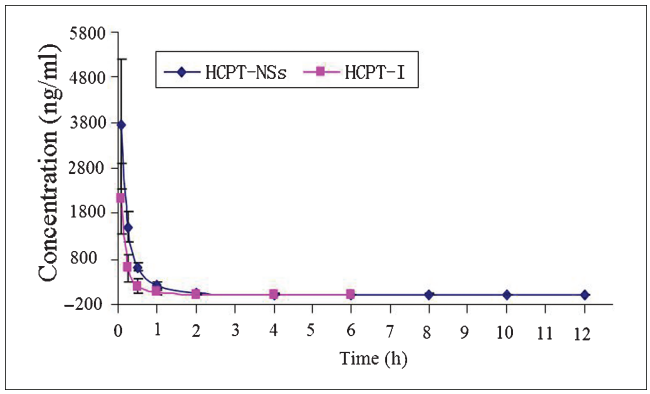

Fig.: Concentration-time curve of HCPT in rat plasma following intravenous administration of HCPT-NSs and HCPT-I at a dose 4 mg kg^{-1} (Data were given as mean ± S.D., n = 6)

The HCPT blood concentration-time curves after intravenous injection of different formulations in rats are shown in the Figure. The HCPT-NSs showed initial higher blood circulating levels than HCPT-I. In fact, the concentration of HCPT-NSs in blood 6 h after intravenous administration was about 7.63-fold than that observed for HCPT-I. After 6 h, HCPT-I was quickly removed from the circulating system and could not be detected. On the contrary, HCPT-NSs exhibited a remarkable delayed blood clearance. It could be seen that the blood concentration of HCPT-NSs remained higher after 12 h. The concentration–time curves for HCPT-NSs and HCPT-I in rats were fitted by the two-compartment model, and their pharmacokinetic parameters are shown in the Table. The result showed that HCPT-NSs could extend $t_{1/2(\beta)}$ of HCPT to 4.40 h. Meanwhile, the AUC increased by 2.36-fold, compared to HCPT-I. Rabinow (2004) suggested that the nanocrystal suspension was cleared more slowly to give a longer $t_{1/2(\beta)}$ and larger area under the plasma concentration curve. This behaviour is consistent with the monocyte phagocytic system (MPS) depot behaviour, resulting in prolonged delivery for the nanosuspension. The HCPT naocrystals, uptaken by MPS, might dissolve slowly in phagocytic cells and release slowly into blood circulation, and remain a longer blood level compared to HCPT-I. In order to further evaluate tumor targeting efficiency of HCPT-NSs, the tissue distribution Table of HCPT in Sarcoma 180 tumor bearing mice is under way.

Experimental

HCPT-NSs and HCPT-Injection (HCPT-I) were used in this experiment. HCPT-NSs or HCPT-I was administered intravenously into the tail vein of rat at a dose of 4.0 mg HCPT eq/kg. Blood samples (0.4 ml) were taken with a heparinized syringe at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 12 h after administration by removing the eyeballs of rats. Plasma was obtained by centrifugation of the blood at 4000 rpm for 15 min. Camptothecin (4 μg/ml, 25 μl) as an internal standard was added into 200 μl of plasma, and vortexed

with 25 µl phosphoric acid for 1 min. The drug and internal standard were then extracted into 1 ml of ethyl acetate by vortex mixing for 3 min. After centrifugation of the mixture at 3500 r min^{-1} for 15 min, the upper organic layer was collected and evaporated to dryness with N_2 at 35 °C. The residue was reconstituted in 50 µl of HPLC mobile phase, vortexed for 3 min and centrifuged before analysis. The resultant sample (20 µl) was analyzed by HPLC.

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