9-Nitrocamptothecin polymeric nanoparticles: cytotoxicity and pharmacokinetic studies of lactone and total forms of drug in rats

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The objective of this study was to evaluate the cytotoxicity and pharmacokinetics of total and lactone forms of 9-nitrocamptothecin (9-NC), an effective antineoplastic drug, after intravenous injection of drug incorporated into poly (DL-lactic-glycolic acid) nanoparticles (NPs). Drug-loaded NPs (9-NC.NP) were prepared by the nanoprecipitation method and examined for particle characteristics and in-vitro release in phosphate buffered saline. The best formulation showed a narrow size with an average diameter of 207 ± 26 nm and a drug loading of more than 33.5%. The drug release profile showed a sustained 9-NC release up to 160 h. For a pharmacokinetic study, the concentration of 9-NC as the lactone form (9-NC.lac) and as the total of the lactone and carboxylate forms (9-NC.tot) in plasma was determined by using reverse-phase high performance liquid chromatography after intravenous administration of 9-NC.NP and a control solution to cannulated Wistar rats. In-vitro cytotoxic activity of 9-NC.NP and control solution was evaluated on the human ovarian cancer cell line (A2780sn) by MTT cell cytotoxicity assay. Results of in-vivo studies showed that NP encapsulation markedly increased the plasma concentration of both lactone and total forms of 9-NC compared with free drug. In comparison with free drug, NPs resulted in 3.63-fold and 5.40-fold increases in area under the plasma concentration-versus-time curve ($AUC_{0-\infty}$) for lactone and total forms of 9-NC, respectively. The values of mean residence time and elimination half-life ($T_{1/2}$) were also significantly higher for NPs than for free drug. The in-vitro cytotoxicity study revealed that the IC_{50} value of NPs decreased 10-fold compared with the drug solution. Prepared NPs described here were considered potentially useful in both stabilizing and delivering 9-NC and enhancing the efficacy of this drug for cancer treatment for which high drug retention in the body, protection from the drug-active lactone form, and gradual drug release appeared to be related. *Anti-Cancer Drugs* 19:805–811 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Recently, increasing attention has been focused on formulating therapeutic agents in biocompatible nanocomposites such as liposomes, nanocapsules, micellar systems, and conjugates. Among the new drug delivery systems, the polymeric nanoparticle (NP) has been considered as a promising carrier of anticancer drugs and has become an important area in cancer nanotechnology [1–4]. Most small molecule chemotherapeutic agents have a large volume of distribution leading to a narrow therapeutic index owing to a high level of toxicity in healthy tissues. Through encapsulation of drugs in a macromolecular carrier, such as NPs, tissue distribution and pharmacokinetics of a drug could markedly be changed [5–7]. Under optimal conditions, the drug is carried within the carriers whereas in circulation it is protected from metabolism and inactivation in the plasma and owing to size limitations in the transport of large molecules or carriers across healthy endothelium, the drug accumulates to a reduced extent in healthy tissues. However, discontinuities in the endothelium of the tumor vasculature have been shown to result in an increased extravasation of large carriers and in combination with impaired lymphatics, an increased accumulation of NP-loaded drug at the tumor [8–10]. Furthermore, more recently, it was reported that NPs could overcome the multidrug resistance phenotype mediated by P-glycoprotein (Pgp) leading to an increase in drug content inside the neoplastic cells. This finding is of great importance for the particular case of camptothecins as acquired resistance to chemotherapeutic agents has already been reported [11].

9-Nitrocamptothecin (9-NC), a lipophilic derivative of camptothecin, is a potent inhibitor of totpoisomerase I and has demonstrated high antitumor activity against

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advanced pancreatic carcinoma, ovarian epithelial cancer, and leukemia. In addition, 9-NC has also been found to inhibit HIV-1 replication and has potential clinical utility in HIV-infection/AIDS [12–14].

However, delivery of this category of anticancer drugs is quite challenging for several reasons. First, camptothecin analogs undergo a reversible, pH-dependent reaction between the active lactone and inactive hydroxy acid forms in biological mediums [15,16]. To reduce this problem, liposomal formulations of camptothecin analogs have been investigated to keep the lactone form protected in the hydrophobic lipid layers [17–19]. Second, owing to cell cycle-specific action of these agents, it is advantageous to expose tumor cells to the drug for a prolonged period [20]. This point is supported by clinical observations that patients refractory to topotecan, a hydrophilic analog of camptothecin, exhibited increased response rates when the drug was administered as a low-dose infusion [21]. Poor water solubility of 9-NC and most other camptothecin derivatives is the third concern for the delivery of these agents. Owing to low aqueous solubility, 9-NC is currently developed and administered orally for the treatment of gastric or pancreatic cancers [13,14]. However, it has been reported that orally administered camptothecins have poor bioavailability and have substantial interpatient variability in systemic exposure, which could result in suboptimal antitumor activity or enhanced risk of toxicity [22–24].

To address these problems in our previous study [25], 9-NC-loaded polymeric NPs were prepared and characterized with regard to morphology, size, drug loading, and in-vitro drug release. Owing to the acidic microclimate of poly (DL-lactic-glycolic acid) (PLGA) [26,27] and stability of 9-NC active lactone form in acidic pHs [15], this polymer was considered a suitable polymeric matrix. For further evaluation of the prepared system, in-vivo pharmacokinetics and in-vitro cytotoxicity activity of 9-NC incorporated in NPs were studied in the present study and the results were compared with free drug solution.

Materials and methods

Materials

9-NC, 99.8% pure, was purchased from Yuanjian Pharmaceutical Technology Develop Co., (Huang Shi, China). Poly (DL, lactide-co-glycolid) (PLGA, 50 : 50 MW 12 000) was obtained from Boehringer Ingelheim Co. (Ingelheim, Germany). Polyvinyl alcohol (PVA, MW 30 000 Da, 87% hydrolyzed) was a gift from Mowiol (Frankfurt, Germany). High performance liquid chromatography grade acetonitril and the analytical grade dimethyl sulfoxide, chloroform, acetone, and perchloric acid (70%, w/v) were purchased from Merck (Darmstadt, Germany). [3–(4,5–dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] MTT were obtained from Sigma-Aldrich (Poole, UK). Drug-free human plasma was obtained from the Central Laboratory of the Blood Transfusion Service (Tehran, Iran).

Preparation and characterization of 9-nitrocamptothecin-loaded nanoparticles

NPs were prepared by the nanoprecipitation method as previously reported [25] and the best formulation was selected by factorial design analysis. Briefly, the procedure was as follows: an exact quantity of PLGA polymer (165.27 mg) and 9-NC (1 mg) was accurately weighted and dissolved in 12 ml acetone. The organic phase was added dropwise into 21.5 ml PVA aqueous solution (1.3%, pH was adjusted to 3 by 0.1 mol/l HCl) and stirred magnetically at room temperature until complete evaporation of the organic solvent. Subsequently, NPs were separated by ultracentrifugation (Beckman, XL-90) at 40 000 rpm and 4°C for 1 h and washed three times with distilled water (pH = 3). NPs were characterized in terms of size, loading, and in-vitro release profile.

Cytotoxicity study

Human ovarian cancer (A2780sn) cell line was purchased from the American Type Culture Collection. The cells were maintained in Dolbecco's modified Eagle's medium cell culture medium supplemented with fetal bovine serum to 10%, Na pyruvate to 1 mmol/l, and penicillin and streptomycin to 50 units/ml and 50 μg/ml, respectively, in 150 cm^2 flask. The cells were incubated at 37°C with a 5% CO_2 atmosphere. Cells were harvested from flasks with 0.25% trypsin/0.03% EDTA.

The cytotoxicity of optimized preparation of the 9-NC NPs against the human ovarian cancer (A2780sn) cell line was studied using a MTT test [28]. Cells were transferred (15 000 cell/well) into 96-well plates and incubated for 24 h.

Nanoparticle formulations with 9-NC concentration of up to 20 μg/ml dispersed in medium were added to cells grown in 96-well plates (about 75% confluence), in three replicates. After 24 h incubation, drug samples were removed and replaced by 200 μl fresh Dolbecco's modified eagle's medium and incubated for 20 min. This medium was replaced by 200 μl RPMI medium and 20 μl of MTT dye solution (10% in phosphate buffer pH = 7.4) was added to each well. After 2.5 h of incubation at 37°C and 5% CO_2, the medium was removed and formazan crystals were solubilized by adding 200 μl of dimethyl sulfoxide and the solution was vigorously mixed to dissolve the reacted dye. The absorbance of each well was read on a microplate reader (Tecan, Austria) at 570 nm. The spectrophotometer was calibrated to zero absorbance using culture medium without cells. The relative cell viability (%) related to control wells containing cell culture medium without drug NPs or solution was calculated by $(A)_{test}/(A)_{control} \times 100$, where $(A)_{test}$ is the

absorbance of the test sample and $(A)_{control}$ is the absorbance of control sample.

IC_{50} values, the drug concentration at which inhibition of 50% cell growth was observed in comparison with that of the control sample, were calculated by the curve fitting of the cell viability data using Prism software.

Pharmacokinetic study

Laboratory animals

Pharmacokinetic studies were performed in male Wistar rats weighing about 200–250 g. The rats were fasted overnight before experimentation and had access to water *ad libitum*. They were divided into four groups: two groups ($n = 6$, in each group) for in-vivo study of the 9-NC.tot and two other groups ($n = 3$, in each group) for 9-NC.lac. All animals were housed in wire cages in a 12-h light–dark cycle for a minimum of 5 days before the beginning of the experiment to allow them to adjust to the new environment.

Surgical procedures

All animal experimental protocols were approved by the animal welfare commission of the Shaheed Beheshti Medical University. The day before the experiment, the rats were anesthetized with an intraperitoneal injection of a ketamine/xylazine solution (80 mg/kg ketamine, 12 mg/kg xylazine). After shaving and disinfecting the ventral cervical skin, a 2-cm incision was made right of the midline with its caudal terminus at the level of the clavicle to expose the right pectoral muscle. Underlying salivary and lymphatic tissues were separated by means of a blunt dissection to visualize the right common jugular vein. The caudal and rostral side of the vein were tied by surgeon's silk to prevent bleeding. A small incision was made using microscissors and the cannula, filled with heparin solution [1.0 ml stock heparin (1000 IU/ml + 19 ml) physiological saline], was inserted into the vein. A sterile polyethylene 20 cannula (PE20) was inserted into the vessel and secured in place with a suture. A 0.5-cm midline skin incision was made between the scapulae and cannula was drawn through the scapular incision. The cannula port was flushed by heparinized physiological saline (50 IU/ml heparin) and sealed with a sterile stainless steel pin 23-gauge blunted needle.

After surgery, the rats were housed individually in cages and allowed to acclimatize for 1 day in a 12-h light/dark cycle before pharmacokinetic study [29].

Drug administration and sample collection

The animals were treated with optimized NPs containing 2 mg/kg of 9-NC. Nanoparticle dispersion was prepared freshly and diluted in physiological saline immediately before administration. Owing to the small size of the NPs, this colloidal dispersion was completely homogeneous without any precipitation during injection and had good syringability and suitable physical stability. For comparison, a pharmacokinetic evaluation of free drug was also performed. 9-NC was dissolved in physiological saline right before the intravenous (i.v.) injection. The formulations (NPs or free drug solution) were injected via the jugular vein. Serial blood samples (0.4 ml) were collected from individual rats through the jugular vein cannula using a 1-ml syringe. After each blood sample, the cannula was flushed with 20 µl of heparinized saline (50 IU/ml heparin). Plasma was separated by centrifugation and stored at $-20^{\circ}C$ until analysis.

A typical blood sampling schedule after i.v. dosing was 0, 15, 30, 60, 120, 180, and 240 min and variously thereafter up to 400 min, depending on the formulation. Drug concentration in plasma as lactone or total form was analyzed by the high performance liquid chromatography method developed in our lab [30].

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by noncompartmental analysis and also by the two-compartmental open model. The two-compartment model can be described by the following biexponential equation:

$$C_t = A\,e^{-\alpha t} + B\,e^{-\beta t},$$

where C_t is the drug concentration (*Y*-axis) at time t (*X*-axis). A and B are the Y-intercepts, and α and β are the apparent first-order distribution and elimination rate constants. Elimination rate constant was estimated by least squares regression of plasma concentration–time data points lying in the terminal log-linear region of the curve. The area under the plasma concentration-versus-time curve (AUC) was calculated using the trapezoidal rule with extrapolation to infinity. Clearance (Cl) was calculated by dividing dose over AUC. Volume of distribution at steady state (V_{ss}) and mean residence time (MRT) were calculated using following equations:

$$V_{ss} = \text{dose.AUMC}/(\text{AUC})^2,$$

$$\text{MRT} = \text{AUMC}/\text{AUC},$$

where AUMC (area under the first moment curve) is the area under the C_t plotted against t from time 0 to infinity [31].

Statistical analysis

One-way analysis of variance was performed to compare the parameters between the 9-NC NPs and free drug solution groups. The level of significance was $P < 0.05$.

Results and discussion

NPs of biodegradable polymers can improve the therapeutic effects and reduce the side effects of the

formulated drugs. 9-NC is a potent antitumor agent and the presence of lactone functionality plays an important role in its biological activity [15,16]. On account of the poor water solubility of the lactone form, its instability in plasma pH, and its having a cell cycle-specific mechanism of action, 9-NC-loaded PLGA NPs were prepared in our earlier study [25]. The particle size was found to be adequate for such an effect; furthermore, the yield and drug content of NP were fairly good. For further investigation, the cytotoxicity of loaded drug and free drug, as well as pharmacokinetic parameters of 9-NC in total and lactone forms were investigated in this study.

Characterization of 9-Nitrocamptothecin nanoparticles

The resulting 9-NC NPs showed the best response to size with an average of 207 ± 26 nm, best recovery of 95%, and optimum loading of about 33%. The release profiles of 9-NC total from the PLGA NPs showed that about 20% of the drug was released over a period of 20 h, followed by a distinct prolonged release up to more than 160 h [25]. The release profile of 9-NC lactone form in phosphate buffered solution (pH 7.4) was also evaluated. The results obviously showed that instead of quick hydrolysis of lactone form, more than 80% in around 20 min [15], our NPs could release this labile form of drug for more than 20 h (data not shown). Recently, a novel liposomal formulation capable of incorporating large amounts of 9–NC was reported by Chen *et al.* [17]. The results of in-vitro release studies in phosphate buffered solution showed that about 50% of the drug was released from the prepared liposomes in less than 3 h.

In-vitro cytotoxicity assay

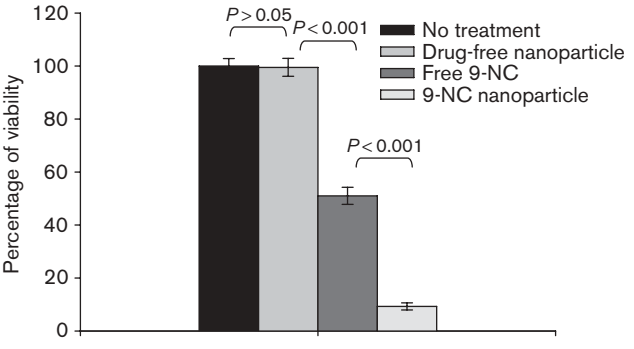
The cytotoxicity of different 9-NC preparations (NP loaded with 9-NC and free drug at the same concentration) was investigated using an ovarian cancer cell line (A2780sn). The results showed lack of any cytotoxic effect of empty NPs and no treatment cells compared with free and loaded drug. 9-NC NPs demonstrated the highest toxicity against the cancer cell line studied. After 24 h, the NP formulation and free drug killed 91% and 49% of cells, respectively. The difference between NPs and free drug formulation was statistically significant ($P < 0.001$) in all studied cases. The IC_{50} values for different preparations of 9-NC were also determined. The IC_{50} of free 9-NC formulation was calculated as 5 μg/ml compared with 0.5 μg/ml in the case of NPs in the ovarian cancer cell line. The NP formulation was about 10-fold more toxic than the free 9-NC in phosphate-buffered saline buffer. Similar results were reported for other anticancer drugs incorporated in NPs [32–35] and a higher cellular uptake of drug-loaded NPs by an endocytosis mechanism rather than by passive diffusion and possible circumvention of the efflux systems, especially Pgps, has been suggested for the observed higher cytotoxic activity [32–35]. For example, the cytotoxicity profile of paclitaxel NPs (PX NPs) was monitored using two different cell lines, U-118 and HCT-15. The results showed that entrapment of paclitaxel in NPs significantly increased the drug brain uptake and its toxicity toward Pgp-expressing tumor cells. It was hypothesized that PX NPs could mask paclitaxel characteristics and thus limit its binding to Pgp, which consequently would lead to higher brain and tumor cell uptake of the drug. IC_{50} values for PX NPs were lower than for Taxol, which may indicate lack of paclitaxel interaction with Pgp when the drug is entrapped in NPs [35]. Based on the recently published report concerning involvement of multiple efflux transporters including Pgp in the efflux of 9-NC [36], the mechanism of circumventing the efflux system by NPs cannot be ruled out.

Besides the abovementioned possible mechanism, rapid hydrolysis of free camptothecins in phosphate buffer and in presence of serum albumin [15,16] could account for higher cytotoxicity of NPs. As the intactness of lactone moiety is structurally important for biological activity [37], the 9-NC NP, which may preserve lactone moiety, was much more effective than free drug in inhibiting cancerous cell growth (Fig. 1). The presence of low microenvironmental pH in PLGA carrier can help to maintain this active form [26,27].

In addition, because of S phase-specific cytotoxicity of camptothecins, it is important to expose cancer cells for a prolonged time to the active lactone form of camptothecins [32]. Therefore, the enhanced antitumor activities of 9-NC NPs may be explained by their ability to maintain a higher portion of active lactone form for a prolonged time, whereas free 9-NC is quickly hydrolyzed into its inactive carboxylate form.

In the study by Yang and coworkers, the antitumor effects against sarcoma 180 solid tumor and plasma concentration time profiles of irinotecan (CPT-11) aqueous solution and

Fig. 1



Cytotoxicity of various preparations of 9-NC at the fixed concentration (5 μg/ml) in ovarian cancer cell line (A2780sn). 9-NC, 9-Nitrocamptothecin.

NP aqueous suspension were compared. CPT-11 aqueous solution was not effective, but NP aqueous suspension significantly suppressed tumor growth [34].

Pharmacokinetic analysis

The mean pharmacokinetic parameters for 9-NC in total and lactone forms of free 9-NC and NPs in rats and the comparisons of pharmacokinetic parameters for both formulations are shown in Tables 1 and 2, respectively. It should be mentioned that according to the studies by Chen *et al.* in rats, the route of administration seems to have a significant effect on the pharmacokinetics of 9-NC, as the elimination half-life and MRT of the drug following oral administration were obviously higher (about twofold) compared with i.v. injection. The elimination half-life and MRT of free drug in our study ($t_{1/2} = 0.83 \pm 0.08$ and MRT $= 1.18 \pm 0.17$) were in accordance with the reported values by Chen *et al.* [38] after oral administration of 9-NC solution ($t_{1/2} = 0.8 \pm 0.3$ and MRT $= 1.4 \pm 0.4$), which were slightly different from those reported in the same study for i.v. administration ($t_{1/2} = 0.5 \pm 0.12$ and MRT $= 0.60 \pm 0.14$). Therefore, owing to the presence of limited reports on the pharmacokinetics of 9-NC, it seems that further investigations are needed to clarify the effect of the route of administration on the pharmacokinetics of 9-NC.

Table 1 Mean pharmacokinetic parameters (mean ± SD) and statistical comparison of the 9-NC.tot pharmacokinetic parameters following i.v. administration of free and loaded nanoparticles to rats

	9-NC.tot		
PK parameter	Free drug	Nanoparticle	*P* value
K (h^{-1})	0.85 ± 0.09	0.29 ± 0.03	<0.0001
$T_{1/2}$ (h)	0.83 ± 0.08	2.45 ± 0.27	<0.0001
C_0 (ng/ml)	571 ± 103	4526 ± 1070	<0.0001
V_{ss} (ml)	767 ± 43	195 ± 59	<0.0001
Cl (ml/min)	10.76 ± 1.64	2.09 ± 0.31	<0.0001
MRT (h)	1.18 ± 0.17	1.56 ± 0.36	<0.05
AUC_{0-t} (ng.h/ml)	713 ± 115	3756 ± 1159	<0.0001
$AUC_{0-\infty}$ (ng.h/ml)	683 ± 144	3692 ± 868	<0.0001
$AUMC_{0-\infty}$ (ng.h/ml)	916 ± 237	6254 ± 2111	<0.0001

AUC, area under the plasma concentration-versus-time curve; AUMC, area under the first moment curve; i.v., intravenous; MRT, mean residence time; 9-NC, 9-Nitrocamptothecin; PK, pharmacokinetic; tot, total.

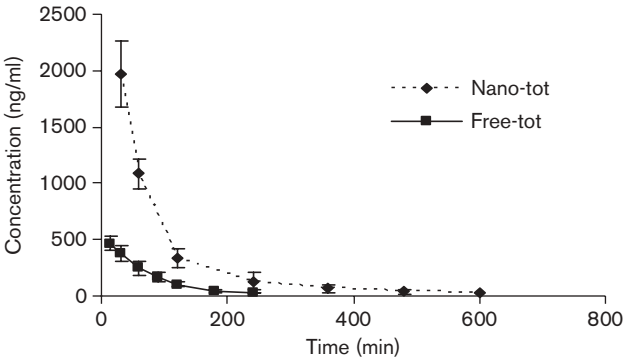
Table 2 Mean pharmacokinetic parameters (mean ± SD) and statistical comparison of the 9-NC.lac pharmacokinetic parameters following i.v. administration of free and loaded nanoparticles to rats

	9-NC.lac		
PK parameter	Free drug	Nanoparticle	*P* value
K (h^{-1})	0.78 ± 0.02	0.31 ± 0.05	<0.001
$T_{1/2}$ (h)	0.88 ± 0.02	2.03 ± 0.05	<0.001
C_0 (ng/ml)	358 ± 5	709 ± 95	<0.005
V_{ss} (ml)	1305 ± 121	725 ± 255	<0.05
Cl (ml/min)	17.79 ± 2.22	4.15 ± 0.98	<0.001
MRT (h)	1.22 ± 0.03	1.56 ± 0.36	<0.001
AUC_{0-t} (ng.h/ml)	404 ± 44	3756 ± 1159	<0.005
$AUC_{0-\infty}$ (ng.h/ml)	445 ± 82	3692 ± 868	<0.005
$AUMC_{0-\infty}$ (ng.h/ml)	532 ± 78	6254 ± 2111	<0.0001

AUC, area under the plasma concentration-versus-time curve; AUMC, area under the first moment curve; i.v., intravenous; lac, lactone; MRT, mean residence time; 9-NC, 9-Nitrocamptothecin; PK, pharmacokinetic.

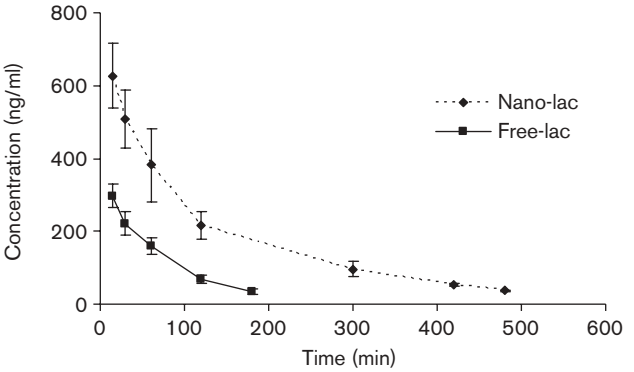
The mean plasma concentration–time profiles of 9-NC.tot and 9-NC.lac following i.v. administration of both formulations (NPs and free drug solution) in rats are shown in Figs 2 and 3, respectively. As shown after i.v. bolus administration of free drug, plasma concentrations of both total and lactone forms of 9-NC declined at a relatively fast elimination rate whereas plasma concentration–time profiles of 9-NC (total and lactone) in NP formulation showed a much slower elimination phase. It is clear that encapsulation of drug in a NP carrier markedly slowed down the elimination phase and resulted in about a 2.95-fold ($P < 0.0001$) and 2.31-fold ($P < 0.001$) increase in the elimination half-life ($t_{1/2}$)

Fig. 2



Plasma concentration–time curve for 9-NC (total form) following bolus intravenous injection of 2 mg/kg of free (free-tot) and drug-loaded nanoparticles (nano-tot) to rats. Each point represents the mean ± SD of six animals. 9-NC, 9-Nitrocamptothecin; tot, total.

Fig. 3



Plasma concentration–time curve for 9-NC (lactone form) following bolus intravenous injection of 2 mg/kg of free (free-lac) and drug-loaded nanoparticles (nano-lac) to rats. Each point represents the mean ± SD of three animals. Lac, lactone; 9-NC, 9-Nitrocamptothecin.

values of total and lactone forms, respectively, compared with free drug. After 8 h, 9-NC was still detectable in plasma following the injection of NPs compared with 3 h for free drug administration (Figs 2 and 3).

The extent of 9-NC distribution was also reduced significantly through NP formulation. Steady-state volume of distribution (V_{ss}) was obviously lower for the carrier-loaded drug (both total and lactone forms) than respective values for the free drug (Tables 1 and 2). It therefore seemed that encapsulation of 9-NC in NPs considerably delayed the kinetics of drug transfer from the plasma to the tissue compartment, which resulted in reduced drug distribution. Considering that the drug release rate from carriers has a high impact on drug distribution, 9-NC release from particles, it seems, was not instantaneous upon administration.

Further pharmacokinetic analysis showed that after administration of NPs, AUC and MRT parameters for 9-NC.tot increased 5.4-fold and 1.32-fold ($P < 0.05$) and for lactone form increased 3.63-fold and 2.42-fold ($P < 0.005$), respectively, compared with free drug (Tables 1 and 2). These results show that prepared NPs were able to reduce the clearance rate of drug and the carrier had obviously limited phagocytosis uptake by the reticuloendothelial system . This system has a major role in removing small foreign particles from blood by coating them with serum components, the opsonins, which act as labels to passively target the NPs to certain phagocytic cells [3,4]. On account of distinct sustaining and retaining of 9-NC lactone active form in plasma, the tumor is expected to be exposed to the active drug form for a much longer time.

Similar results were obtained in the pharmacokinetic study of camptothecin solid lipid NP (CA-SLN) by Yang and coworkers [34]. The AUC/dose and the mean residence times (MRT) of CA-SLN were significantly higher than those of drug solution. In addition, incorporation of 7-ethyl-10-hydroxycamptothecin (SN-38), which has marked antitumor effects on many types of tumors, into NPs improved the stability of the lactone ring in serum-containing media. The plasma half-life of SN-38 was greatly prolonged by incorporation into the NPs [39].

Reddy and Murthy evaluated and compared the pharmacokinetics and tissue distribution of doxorubicin (Dox) after intravenous and intraperitoneal injection of free drug solution and drug-loaded NPs. For both routes of injection, the $T_{1/2}$, MRT, and AUC of drug after the administration of Dox NPs were significantly higher and the clearance (Cl) was lower than the expected values for Dox-free drug solution. The bioavailability of Dox in NP form was greatly enhanced (about 2-fold) compared with the solution, which is expected to improve the therapeutic efficacy of drug and reduce the Dox-associated systemic toxicity, especially cardiotoxicity [40].

Although the prepared NPs in our study with an average particle size of 207 nm showed distinctly favored pharmacokinetic parameters compared with free drug, however, NPs with a hydrophilic shell or smaller particle size (less than 100 nm), which may escape more easily from the reticuloendothelial system, might have more residence time in the body.

Taken together, on the basis of the results obtained, increased efficacy compared with free drug in a cancerous cell model, prolonged half-life of the active (lactone) form in plasma, and markedly improved pharmacokinetic parameters were achieved by the formulation of 9-NC as polymeric NPs.

Conclusion

In this study the NP formulation of 9-NC was investigated in terms of in-vitro cytotoxicity and in-vivo pharmacokinetics of total and lactone forms of the drug. The NPs prepared were characterized by efficient drug loading, sustained drug release in the buffer solution, prolonged circulation in rat plasma, and better efficacy against a resistant model of human ovarian cancer cell line (A2780sn). Therefore, PLGA NPs may be a promising sustained release and drug targeting system for lipophilic analogs of camptothecin and are expected to allow a reduction in dosage and a decrease in systemic toxicity. Study of the biodistribution and antitumor activity of the 9-NC-loaded NPs in animal models could provide further support for the therapeutic advantage of the formulation prepared.

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