

Vincristine-Dextran Complex Loaded Solid Lipid Nanoparticles for Drug Delivery to the Brain

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Abstract—The purpose of this work was to inspect the potential of vincristine-dextran complex loaded solid lipid nanoparticles for drug delivery to the brain.

The nanoparticles were stained with a fluorescence dye and their plasma pharmacokinetic and brain concentrations were investigated following injection to rats.

The result revealed a significant improvement in the plasma concentration profile of the SLN injected animals as well as a sharp increased concentration in the brains.

Keywords—Brain, Coumarin-6, Nanoparticles, SLN.

I. INTRODUCTION

BRAIN tumors are considered the most fatal and hard to cure malignancies. According to the National Cancer Institute each year in the United States, more than 35,000 people are diagnosed with primary brain tumors[1]. In addition more than half of all intracranial malignancies in adult population are metastases from systemic malignancies. Breast, ovarian and small cell lung cancers are among the most metastatic cancers to the brain[2], [3]. One of the most important obstacles which hamper the effective treatment of brain disorders is the blood-brain barrier (BBB). This sophisticated system is composed of special tight junctions and a highly active P-glycoprotein (P-gp) efflux system and is in charge of controlling the brain microenvironment by actively controlling the passage of molecules between blood and the brain extracellular fluid (BECF). Unfortunately most of the anticancer drugs are effectively expelled from the BECF by this system and cannot reach their minimum effective concentration.

The situation in other brain diseases is the same and in many cases the poor penetration of drug molecules to the brain leads to therapy regimens failure[4].

Solid lipid nanoparticles (SLNs) are biocompatible and versatile drug nanocarriers which in several studies had shown superior efficacy in drug targeting to the brain [5]-[8].

Also some of the popular surfactants in SLN production Tween 80(T80) and Vitamin E TPGS (TPGS) were found to be P-glycoprotein inhibitors [9], [10].

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The lipophilic core of these nanoparticles can easily incorporate fat soluble drug molecules with high encapsulation efficiencies and also prolong the drug release.

For the hydrophilic molecules the encapsulation efficiency in most cases are lower since in almost all of the SLN fabrication methods at least in one stage the drug molecules face water and molten lipid phases at the same time and tend to partition in aqueous fraction of the formulation. Using Counter ions is a practical strategy to incorporate hydrophilic ionic molecules into lipophilic matrices [11]-[13].

Vincristine Sulfate (VC) is a very potent anticancer drug and is being used in many primary and metastatic brain cancers while it is a substrate of P-gp and is effectively expelled from the CNS [14],[15] and cannot reach high levels in the CNS [15], [16].

In a previous study we developed and optimized a VC loaded SLN formulation using dextran sulfate as the counter ion to diminish the high charge and hydrophilicity of Vincristine Sulfate. The invivo studies after injection to rats demonstrated a significant improvement in the pharmacokinetic profile (significant increase in the AUC of the plasma-time profile and mean residence time of the drug in the body)[17].

To assess the ability of SLNs to penetrate into the CNS, two SLN formulations with different surface coatings (either T80 or TPGS) were made by the above mentioned optimized method. The SLNs were stained by coumarin-6 (a fluorescence dye) and their brain permeability potential was evaluated over a 6 hour time period.

Coumarin-6 was selected because of limited leak from the SLNs due to high lipophilicity and very high sensitivity of detection by fluorescence HPLC (LOQ > 10 pg/ml). Also the dye itself has a poor penetration into the brain and the elevated levels in the brain can be regarded as the ability of the SLNs to penetrate into the brain [18].

The stained SLN formulations with T80 or TPGS coatings were injected to adult male Wistar rats via tail veins and plasma and brain concentration of Coumarin-6 was measured by the aid of HPLC coupled with a fluorescence detector.

II. MATERIALS AND METHODS

2.1. Materials

Cetyl palmitate (CP) and T80 were from Merck (Germany), VC, Coumarin-6, and Dextran sulfate were from Sigma Aldrich (Germany), Amicon Ultra-15 ultra centrifuge tubes was from Millipore (United States). All other chemicals were either reagent or HPLC grade, and used as received.

2.2. Preparation and staining of the SLNs

SLNs were fabricated by microemulsion technique using the optimized amounts of the Lipid (CP), Surfactant (T80 or TPGS) found in our previous study. Briefly Coumarin-6 (4mg) and VC (20mg) were dispersed in molten CP (950 mg) by magnetic stirring in 80°C. DS (16 mg) and T80 (660µl) or TPGS (720mg) were dissolved in 10 ml hot water (80°C). The aqueous solution was then added dropwise to the molten lipid fraction, the resulting microemulsion was sonicated (14w, 10 min, 80°C) using the ultrasonic probe sonication system (Misonix, NY, USA). SLNs were then formed by dispersing this hot nanoemulsion into 10 ml of cold water (2-3°C) under mechanical stirring.

2.3. Particle size and Zeta potential determination

Particle size distribution, poly dispersity index (PDI) and Zeta potential of SLNs were measured by Photon Correlation Spectroscopy (PCS) (Zetasizer nano ZS, Malvern Instruments, Worcestershire, UK). Before the experiment, all samples were diluted ten times with RO water to avoid interparticle scattering.

2.4 AFM investigation

Morphology of the nanoparticles was evaluated by atomic force microscopy with Rectangular Cantilever and conical tip (Dualscope/ Rasterscope C26, DME, Denmark) working in non contact mode. Diluted samples were dried and mounted on a cover slip and topography images were generated from 50 × 50 µm areas with nominal resolution of 0.1 nm.

2.5. Entrapment Efficiency (EE) calculation

Unloaded coumarin-6 was concentration was measured after separating nanoparticles from the aqueous phase by ultrafiltration and EE was inversely calculated based on free drug concentration in aqueous media.

Briefly 500µl of SLN formulation was transferred to Amicon® Ultra-15 tubes and were centrifuged at 6000g and in 5°C for 15 min (Sigma 3k30, Sigma Laboratory, Osterode, Germany). The free coumarin-6 concentrations in the filtrate were analyzed by HPLC.

2.6. In vitro leakage study

Release study from the SLNs was performed to evaluate the leakage of the dye from the formulation. 10 ml of freshly prepared SLN dispersion was placed in a dialysis bag (molecular weight cut-off of 12000 D) and the two ends of the bag were clamped and was soaked in 800 ml of isotonic phosphate buffer solution (pH 7.4) as the dissolution medium. Samples at fixed time intervals were withdrawn and replaced with fresh medium to maintain the sink condition. The whole system was closed to prevent evaporation and was kept in a thermostatic horizontal shaker (Heidolph Unimax 1010, Schwabach, Germany) at 37°C

2.7. Chromatographic procedure

As the chromatography apparatus a smartline Knauer HPLC system (Berlin, Germany) with a C8 (Nucleodor®) analytical column(4.6 mm X 250 mm - 5µm particle size)

coupled with a Shimadzu RF-10Ax1 fluorescence detector (Tokyo, Japan) was utilized.

The excitation and emission wavelengths were set at 460nm and 500nm respectively and the mobile phase was a mixture of 90% methanol, 9.2% water and 0.8% acetic acid glacial with a flow rate of 1.2 ml/min.

2.8. Coumarin-6 extraction

To extract Coumarin-6 from the invivo samples; 500 µl of plasma or 500mg of brain homogenates were transferred into a glass tube and coumarin-6 was extracted by liquid-liquid extraction method with 1 ml diethylether (four times). The tubes were centrifuged and the organic layer was then separated, collected and evaporated under stream of nitrogen. The residue was then reconstituted in the mobile phase and sonicated for 5 minutes in an ultrasonic bath and then centrifuged for 5 minutes at 6000g. The supernatant was then injected into the HPLC system.

2.9. In vivo investigation

24 male Wistar rats weighing 220 ± 30 grams were randomly divided to six groups in which three formulations namely CP-T80 SLN, CP-TPGS SLN, and coumarin-6 solution (200µg/ml in propylene glycol) as the control group was each injected to two groups at the dose of 200µg/kg via tail vein. The animals had free access to food and water and were handled according to the ethical animal care committee of Tehran University of medical sciences guidelines.

The first group of each formulation was blood sampled 15, 30, 60 m post injection from the tail veins. The first groups were sacrificed at 60 minutes by decapitation following complete anesthesia with ether. The procedure was carried out isolated from all other conscious animals.

Blood sampling was continued from the second group of each formulation at 120, 240, 360 m of and finally were sacrificed at 360 minutes with the same procedure.

The blood samples were transferred to heparinized tubes and were centrifuged at 4000g to separate the plasma. The brains also were taken out immediately after decapitation and were washed twice with cold PBS solution to minimize the residual blood in the brain.

2.10. Statistical analysis

The plasma and brain coumarin-6 concentration data were compared by one-tailed Student's *t*-test and *P* value of < 0.05 was considered as statistically significant.

III. RESULTS

3.1. Size distribution and Zeta potentials of SLNs

Since the fabrication method and the formulation parameters were optimized in our previous studies the SLN formulations were had a narrow size distribution (PDI < 0.2) and Z average size of 100 ± 4 (Fig 1).

The Zeta potentials of the Formulations were slightly negative and approximately -10mV.

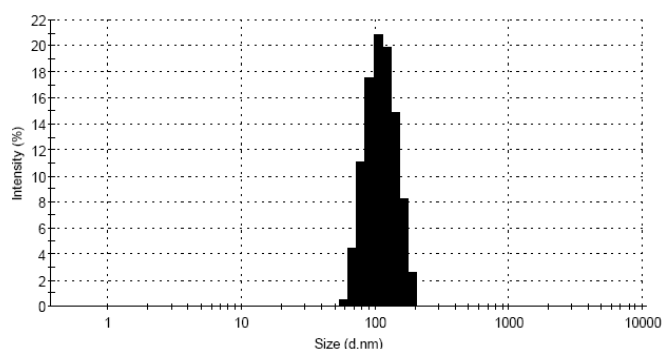


Fig. 1 Size distribution of SLN samples

3.3 AFM topography results

Fig 2 shows the AFM topography image of T80 coated SLNs. The outcome is in agreement with PCS data where the majority of nanoparticles were of a uniform size of about 100nm. The situation for TPGS coated nanoparticles was also similar.

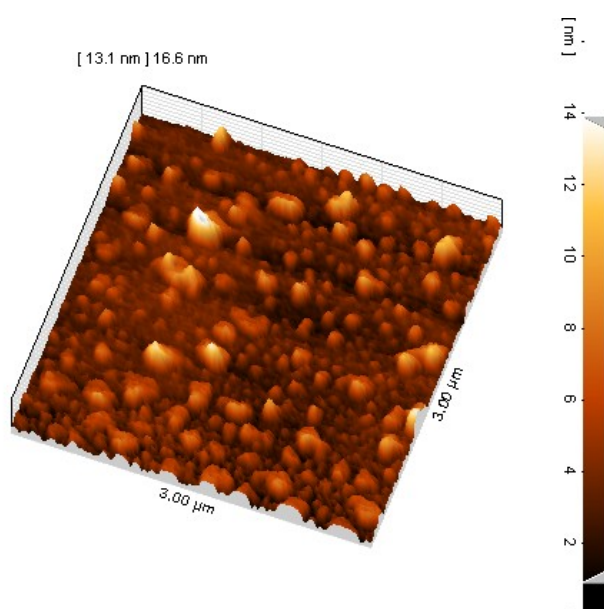


Fig. 2 AFM topography image of SLN samples

As it is apparent in Fig 2; the height of the nanoparticles in topography images is lower than expected; this phenomenon has been reported before for SLNs and other nanoparticles as well. One explanation would be the flattening of SLN or other nanoparticles of soft matter due to gravitational force or the pressure of tip over the soft nanoparticles [19].

In addition to the tip may be submerged into the soft surface of SLNs and result in underestimation of particle height[20].

Another reason may be that the AFM investigations were performed on dry samples while in the PCS measurement the hydrodynamic radius of the SLNs in water was calculated and this difference is related to the dehydration of nanoparticles [21].

3.4. Entrapment Efficiency (EE)

Since the coumarin – 6 is very soluble in molten CP, the entrapment efficiency in SLNs was nearly perfect. In fact at least 98% of coumarin-6 used in the formulation was incorporated into SLNs.

3.5. *In vitro* leakage study

The leakage of coumarin-6 was negligible throughout the study. Indeed less than 2 percent during the 24hr was leaked from the formulation which can be related to the unloaded fraction of coumarin-6 (see 3.4). Therefore the dye would not leak from the formulation during the *in vivo* study and the coumarin content in plasma and brain tissue can be correlated to the SLN (and subsequently loaded VC) concentration *in vivo*.

3.6 Chromatographic procedure

A partial validation was done for the procedure. The lower limit of quantification (LLOQ) was calculated as 10 pg/ml and the linearity of response was satisfactory ($R^2 = 0.9999$) over the range of 50ng/ml to 10pg/ml.

The relative standard deviation for inter-day and intra-day repeated injections were 3.0% and 4.7% respectively and the runtime was about 6 minutes.

3.7 Coumarin-6 extraction

Regarding the high solubility of the coumarin-6 dye in diethylether the extraction from plasma and brain tissue was very good. In fact the extraction recoveries from plasma and brain homogenates were 93% and 86% respectively.

3.8 *In vivo* studies

Fig 3 illustrates the comparative plasma time profile of the the Coumarin-6 stained SLN formulations and coumarin-6 solution following injection to rats. The plasma concentrations at any time points were significantly higher in the SLN administered groups compared to the solution injected group.

The T80 coated SLNs resulted in slightly higher (but statistically insignificant) plasma concentrations compared to TPGS SLNs.

For both SLN formulations a sharp and significant increase in the AUC was observed compared to the solution formulation.

Also the coumarin-6 solution was extensively cleared from the plasma but the SLN formulations showed increased mean residence times.

The coumarin-6 brain content of CP-TPGS, CP-T80, and Solution administrated groups after 60 and 360 minutes are presented in Fig 4.

In both checkpoint times the brain content for T80 coated SLN formulation was significantly higher compared to TPGS coated SLNs and Solution injected groups.

The TPGS coated SLNs were also able to produce higher brain levels in comparison to Solution formulation.

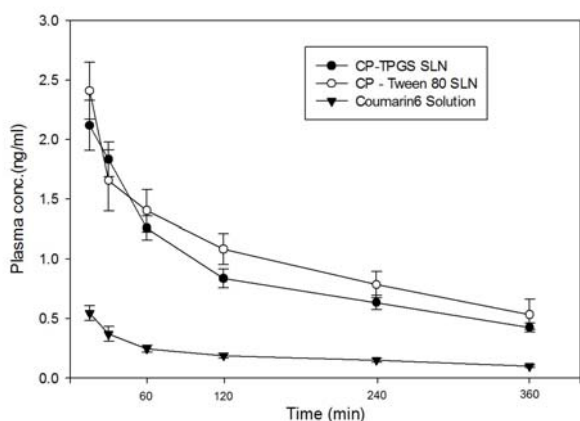


Fig 3. Comparative plasma concentration profile of SLN formulations and coumarin-6 solution.

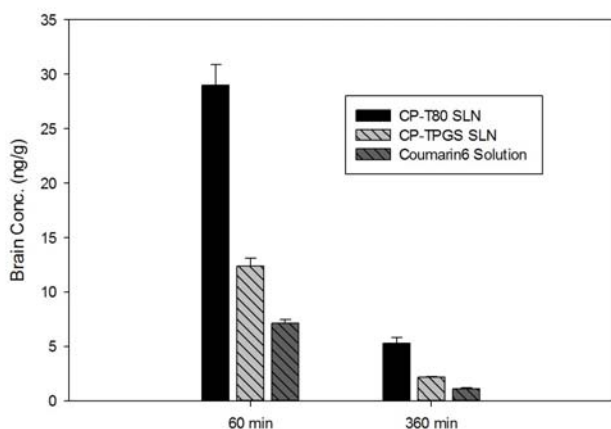


Fig 4. Coumarin-6 brain concentration following injection to rats (200 µg/kg)

IV. DISCUSSION

In our previous study the VC loaded SLN formulation were able to significantly increase the AUC of the plasma – time profile curve of VC (more than 3 fold) as well as lowering the total clearance of VC to one third compared to VC solution.

Also a noticeable increase in the mean residence time for the SLN formulation was observed beside significantly higher VC concentrations were found for liver and spleen.

The brain tissue had also detectable amounts of VC. But due to low sensitivity of HPLC-UV detection quantitative measurement was impossible

In this study we stained the nanoparticles with a fluorescence dye (coumarin-6) and followed the fate of the nanoparticles in plasma and brain tissue.

The coumarin-6 proved to be an excellent tool to investigate the brain permeability of nanoparticles.

Since the dye can be incorporated into lipophilic matrix of the SLNs and would not leak easily. Also very sensitive detection with the fluorescence detectors would help to trace

very low concentrations of in vivo samples and the introduced extraction procedure had excellent extraction recoveries.

Both SLN formulations increased the AUC and MRT extensively but the T80 coated SLNs had a better ability to transfer their content to brain tissue. According to Muller et al the surface coating of the SLNs has a great impact on the fate of the nanoparticles in vivo. They stated that the Tween 80 coated nanoparticles had the highest accumulation in the brain due to a preferential opsonisation pattern (highest apo E / apo C II) [7].

V. CONCLUSION

The SLNs are versatile nanocarriers and are able to improve the pharmacokinetic profile of the incorporated drugs. Also the Tween 80 Coated SLNs due to the preferential opsonisation pattern have the tendency to accumulate in the brain tissue.

These nanocarriers can be formulated to incorporate lipophilic and to a lesser extent hydrophilic drug molecules and seen promising as efficient tools in drug delivery to brain.

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