NANOSPHERIC CHEMOTHERAPEUTIC AND CHEMOPROTECTIVE AGENTS

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Introduction

Paclitaxel (Taxol®) is used widely for the treatment of breast, ovarian, non-small cell lung cancer, prostate, and other types of solid tumor cancers.1 Paclitaxel (PTX) is only sparingly soluble in water, and its intravenous administration depends on the use of Cremophor® EL (polyethyleneglycol castor oil, CEL), to obtain a sufficiently concentrated solution. The use of CEL increases patient toxicity and can lead to clinically important adverse effects, including acute hypersensitivity reactions and peripheral neuropathy.2

To improve the therapeutic potency of paclitaxel and to overcome the toxicities associated with Cremophor® EL, we have developed a unique tyrosine-derived nanosphere system based on a biodegradable, non-cytotoxic polymeric architecture (Scheme 1). This system is intended to improve delivery of a wide range of therapeutics to tumors while eliminating the adverse side-effects of other drug delivery systems. In addition, this formulation might open new avenues for adjunct therapies such as simultaneous administration of several hydrophobic anticancer drugs with different mechanism of activity.

Nano-PTX

Core-forming Nanosphere vehicles: anti-tumor activity, pharmacokinetics and biodistribution

Aim: To evaluate the potential of tyrosine-derived nanospheres as drug delivery vehicles: anti-tumor activity, pharmacokinetics and biodistribution of paclitaxel-loaded nanospheres in human breast tumor xenograft in mouse model.

Results and Discussion

Process steps for formation & purification of drug-loaded nanosphere formulation (ave. 70 nm):

Figure 1. Drug binding efficiency as function of hydrophobicity of the drug and hydrophobicity of the core-forming tyrosine-derived oligomers (HPLC analysis).

The extent of drug hydrophobicity and the affinity of the loaded drug with the core-forming oligomer are the overriding factors for the sufficient drug binding using our nanospheres.

For the anti-tumor drugs investigated herein, there is an optimum degree of hydrophobic properties of the core-forming oligomer that is attained with the octyl ester of the oligo(DTR-XA) hydrophobic block.

In Vitro Cytotoxicity

Figure 2: Metabolic activity of KB cervical carcinoma cells exposed to tyrosine-derived nanospheres prepared in PBS (MTS colorimetric assay).

Figure 3: Effectiveness and non-toxicity of PTX-nanosphere formulation as compared to the Cremophor® EL (CEL) formulation in MDA-MB 435 breast cancer cells (MTS assay, 72 h).

In the investigated copolymer compositions (A) and DTO-SA/SK nanosphere concentration range (B), no significant decrease of the cell metabolic activity of KB cervical carcinoma cells was detected confirming that these nanospheres do not induce any short-term cytotoxicity.

Table 1. The IC50 values of paclitaxel (PTX) delivered via nanospheres vs. DMSO to KB human carcinoma cell (MTS assay, 72 h).

<table>
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<tr>
<th>Dose</th>
<th>PTX via DMSO</th>
<th>PTX loaded DTO-SA/SK</th>
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<tr>
<td>ng/mL</td>
<td>4.3 ± 0.1</td>
<td>0.008 ± 0.002</td>
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Tyrosine-derived nanospheres provide substantially enhanced delivery of paclitaxel to carcinoma cells in vitro.

Anti-Tumor Efficacy

Figure 5: Anti-tumor activity in NCR nu/nu mice bearing subcutaneous MDA-MB-435 breast cancer xenografts. Nanospheric PTX (N=6) were administered via tail vein injections using a dose of 15 mg/kg on a q2d x 5 schedule. As a control, CEL-PTX (N=7) and saline (N=7) were administered to a separate group of xenografted mice using an identical dose and schedule. Tumor volume was measured on two dimensions by length and width.

Using the T-C method,4 the growth delay was calculated to be 244% for PTX and 181% for Nano-PTX.

Tyrosine-derived nanospheres containing paclitaxel exhibit anti-tumor activity in a breast cancer xenograft model that is similar to an equivalent dose and schedule of clinically used formulation of CRL-paclitaxel.

Biodistribution (Preliminary Data)

Figure 6: PTX biodistribution in xenograft-bearing mice.

Nanospheric PTX and CRL-PTX were administered via tail vein injections using a dose of 15 mg/kg. At the specified times 0.5, 1, 3 and 24 h after the i.v. injection mice (N=6) were euthanized using carbon dioxide gas, and blood and tissues were collected. PTX was extracted by tissue homogenization and NaOH extractions. PTX quantification was done by HPLC with calibration standards ranged from 0.025 to 25 ng/mL.

This initial test revealed that in general, accumulation of PTX is comparable in all tested tissues regardless of administration vehicle: rapid and significant uptake (0.5-1 h) and very low concentrations in plasma at 24 h.

The main difference in PTX distribution (24 hours) is in the lung tissue: 43% of PTX delivered via CRL-PTX remained in lungs, while no PTX administrated via CEL was detected.

To show further advantages of tyrosine-derived nanospheres to CRL, pharmacokinetic studies and evaluation of nanospheres ER effect are under investigation.

Pharmacokinetics (Preliminary Data)

Table 2. Plasma paclitaxel pharmacokinetic parameters after administration of a 15 mg/kg dose of nano-PTX. *PK values of plasma paclitaxel delivered via CRL are taken from the literature (ref. 6).

<table>
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<tr>
<th>Dose (mg/kg)</th>
<th>t1/2 (min)</th>
<th>AUC (ng*min/mL)</th>
<th>Vc (mL/kg)</th>
<th>Vss (mL/kg)</th>
</tr>
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<tr>
<td>Nano-PTX</td>
<td>11.25</td>
<td>2013.5 ± 5.43</td>
<td>93 ± 0.5</td>
<td>179.7 ± 24.6</td>
</tr>
<tr>
<td>CRL-PTX</td>
<td>9.69</td>
<td>24.61 ± 0.0082</td>
<td>6.85 ± 33.94</td>
<td>4.6 ± 0.1</td>
</tr>
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</table>

Based on PK parameters and using historical CRL-PTX data, nano-PTX in murine plasma has a larger volume of distribution, higher clearance, and longer half-life than CRL-PTX. The AUC of Nano-PTX is also less than that observed with CRL-PTX. These results may relate to cromerind-induced entrapment of paclitaxel in the blood circulation.

Summarize

Tyrosine-derived nanospheres offer the potential for effective parenteral delivery of a wide array of hydrophobic drugs with reduced cytotoxicity problems commonly exhibited by surfactant-based drug delivery systems.

In a breast cancer xenograft model, paclitaxel-nanospheres exhibit no toxicity and prevent tumor growth similar to clinically used CRL-PTX.

The evaluation of relative efficacy and potential synergies of nanospheres containing both anti-tumor drugs and other chemotherapeutic and chemopreventative drugs such as analogues of vitamin D3 is currently under investigation.

References:

Acknowledgements:

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