Development of a combinational drug-loaded nanoparticle for improving paclitaxel uptake into multidrug resistant breast cancer cells

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ABSTRACT SUMMARY
Paclitaxel have been used in the treatment of wide range of cancers, but its entry into cancer cell is restricted by p-glycoprotein (p-gp) efflux. A potential drug-drug interaction exists between paclitaxel and verapamil because both drugs are metabolized by the cytochrome P-450 system and verapamil can inhibit p-gp efflux of paclitaxel. Hence, paclitaxel was loaded in solid lipid nanoparticle (SLN) and then surface of these SLNs were modified with hydroxypropyl-β-cyclodextrin (HPCD) to load verapamil into SLN. These nanoparticles were characterized by analysis of particle size, zeta potential, entrapment efficiency, in vitro drug release, cytotoxicity, and cellular uptake against breast cancer cells. These particles were designed to release verapamil and paclitaxel, sequentially.

INTRODUCTION
The limitation of chemotherapy in cancer patients is often due to the development of simultaneous resistance to multiple drugs. Some tumors show intrinsic resistance to drugs and others acquire drug resistance with exposure to unrelated drugs. Paclitaxel is an anticancer agent used in the treatment of a wide range of tumors including breast, lung, prostate, ovarian, and pancreatic cancers. Although paclitaxel (PTX) is the drug of choice for the treatment of breast cancer, its use in breast-cancer is limited due to the restricted uptake of the drug by p-gp efflux. Verapamil (VP) is known to a p-gp inhibitor. Some trials has been conducted the combination of cancer drug and p-gp inhibitor.

SLN is well tolerated in living systems since they are constructed from physiological compounds. Therefore, SLNs are easily metabolized. Consequently, they play an important role as drug delivery systems for intravenous, peroral, parenteral, pulmonary or ocular administration.

HPCD is more water-soluble than the parent molecule, β-cyclodextrin, and has hydroxypropyl-ester groups attached to the hydroxyl groups in position 2. In addition, HPCD has known to form inclusion complex with many compounds, which prevents the oxidation of oils and involatile flavors, and solubilizes insoluble compounds.

Therefore, the aim of this study was to co-loading of VP and PTX in SLNs using HPCD to achieve higher cellular uptake by inhibition of p-gp.

EXPERIMENTAL METHODS
We have designed VP and PTX co-loaded SLN to prepare highly uniform and stable nano-sized particles with sustained release of two drugs for enhancing cellular uptake of PTX in MCF-7/ADR cells. So, we designed formulations with variation such as amounts of VP and whether HPCD was used (Table 1).

Table 1. Composition of SLNs.

<table>
<thead>
<tr>
<th>Formulation Unit (mg)</th>
<th>PSV1</th>
<th>PSV2</th>
<th>PSV1</th>
<th>PSV2</th>
<th>PSV1</th>
<th>PSV2</th>
</tr>
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<tbody>
<tr>
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<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Verapamil</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Poloxamer 188</td>
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<td>75</td>
<td>75</td>
<td>75</td>
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</tr>
<tr>
<td>Lecithin</td>
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<td>75</td>
<td>75</td>
<td>75</td>
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</tr>
<tr>
<td>HPCD</td>
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<td>400</td>
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</tr>
</tbody>
</table>

And, Figure 1 shows schematic diagram of the preparation method for various type SLNs. PSV was made by using cyclodextrin for inclusion of verapamil. PVSV was made by same method except for co-loading of VP and PTX. PVS was made by co-loading of VP and PTX in lipid matrix without HPCD.

RESULTS AND DISCUSSION
Particle sizes of PVSs were slightly smaller than those of PSVs and PVSVs (Figure 2). However, polydispersity index of PVSs were larger than those of other SLNs. The mean diameters of all of SLNs were above 350 nm. And, series 1 of PSV, PVS or PVSV showed smaller size than that of series 2 of PSV, PVS or PVSV. The addition of VP-HPCD inclusion complex, the particle size had
increasing tendency due to coating surface area. These results were due to competitive encapsulation of VP and PTX. Both VP and PTX are hydrophobic drugs. But, they have different molecular weight and log P value. So, particle size variations were different in different batches. It was worth to note that the co-loading two different drugs in solid lipid on the variation in the particle size of SLNs.

Figure 2. Particle size and polydispersity index of different SLNs (mean±S.D., n=3).

No significant difference in zeta potential was observed with PSVs and PVSVs (Figure 3). However, the absolute value of zeta potential of PVSs significantly increased in comparison with other SLNs. The encapsulation efficiency of VP and PTX in PVSs were lower than other SLNs coated with HPCD. The E.E (%) of VP and PTX in PSVs and PVSVs were a range of 60~70%. But, encapsulation efficiency (%) of VP and PTX from PVSs were only about 20% or 30%, respectively.

Figure 3. Zeta potential values and encapsulation efficiency (%) of different SLNs (mean±S.D., n=3).

Cumulative drug release profiles were obtained by representing the cumulative percentage release of drug with respective to the amount of drug encapsulated in the SLNs (Figure 4). Release of PTX in all formulations showed over 60% in 24 hours. In case of PSV1 and PVS1, the release of VP was higher than that of paclitaxel in 12 hours. While the release of VP from PSV1 in 2 h showed about 40%, cumulative amount of VP was only 60% in 24 h. But, the release of VP from PVSV in 2 h was about 20% and release of VP was 67.3±4.4% in 24 h. So, PVSV1 was expected to have a sustained p-gp inhibition effect in MCF-7/ADR cells. This result was explained as follows. First, the hyrophobicity of a drug was in the order of PTX > VP. Second, the reverse order was tenable for the binding force of a drug in the lipid matrix. Therefore, a relatively large portion of VP located near the external region of SLNs. The release of a drug from the SLN can be influenced by the nature of the lipid matrix, surfactant concentration and production parameters.8,9

Figure 4. In vitro release of paclitaxel (PTX) and verapamil (VP) from different SLN formulations (A) PSV1, (B) PVS1, and (C) PVSV1 in 0.5% tween 80 solution (n=3).

CONCLUSION

For increasing cellular uptake of PTX into MCF-7/ADR cells, VP and PTX co-loaded SLNs were prepared and evaluated by the physicochemical characterization and release studies. Among SLNs, PVSP showed optimal release profile of VP and PTX.

REFERENCES

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