Preparation, Characterization and Evaluation of a Liposomal Formulation for Co-Delivery of Paclitaxel and Lapatinib

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ABSTRACT SUMMARY
A liposomal formulation for co-delivery of paclitaxel and lapatinib was prepared and characterized. The applied method for preparation was thin film hydration. Its in vitro toxicity against Sk-br-3 cell line was studied to compare the cytotoxic effect of liposomal formulation with individual and also combination drugs as well.

INTRODUCTION
Paclitaxel is one of the most promising anticancer agents isolated from the bark of Pacific Yew tree (Taxus brevifolia). Its unique mechanism of action is stabilizing microtubules by inhibiting their depolymerization, thus stopping cells at the end of G2 mitotic phase. Paclitaxel is approved as the first line treatment in breast and ovarian carcinomas. It has also acceptable antitumor activity against non-small cell lung cancer, head and neck tumors, Kaposi’s sarcoma and urologic malignancies.¹ Its broad spectrum of clinical use is accompanied by some problems including poor water solubility, low therapeutic index and the frequent emergence of multidrug resistance. Taxol® is a commercial formulation of paclitaxel containing Cremophor EL® as the solubilizing agent. The necessary amount of Cremophor needed for delivering the required dose of paclitaxel is significantly higher than other pharmaceutical formulations, thus causing neurotoxicity, nephrotoxicity and severe hypersensitivity reactions. Although premedication with corticosteroids and antihistamines is used clinically to prevent these side effects but milder reactions still occur in patients receiving Taxol® containing regimens.² Another problem with paclitaxel is occurring Multi Drug Resistance (MDR) caused by increasing drug efflux predominantly via ATP-Binding Cassette (ABC) superfamily receptors.

Lapatinib is a reversible dual tyrosine kinase inhibitor which targets both EGFR and HER2 receptors. Studies indicate that lapatinib has inhibitory function against ATP-binding cassette receptors, thus can sensitize resistant cancer cells to chemotherapeutic agents including paclitaxel. Liposomes are carriers with attractive characteristics which include but not limited to: biocompatibility, improvement of pharmacokinetic and drug release, increase therapeutic efficacy and the potential to incorporate more than one agent. These features convert them to ideal carriers for lipophilic drugs such as paclitaxel.³

The objective of this study is to develop a novel liposomal formulation for co-delivery of paclitaxel and lapatinib in order to overcome paclitaxel associated MDR.

EXPERIMENTAL METHODS
Liposomes were prepared by thin film hydration method. Briefly the lipophilic contents (DPPC, lecithin, cholesterol, paclitaxel and lapatinib) were dissolved in chloroform with different compositions and molar ratios for finding the optimum composition. The resulted film was vacuumed overnight under nitrogen flow to remove traces of chloroform. The homogeneous thin lipid film was hydrated with sucrose 10% by connecting to rotavaporator at 60°C and 90 rpm after adding 1 gram of small glass beads without applying vacuum. Obtained liposomal colloidal suspension was sonicated with probe sonicator.
(0.5 cycles and 75 power) for 10 minutes. The formulation was left at ambient temperature about 1 hour to be stabilized then it was centrifuged about 20 minutes to separate free drug form liposomal formulation. The size distribution and zeta potential were determined by Malvern Zetasizer. Differential scanning calorimetry measurements were performed using a differential scanning calorimeter (DSC). The incorporation efficiency was determined by HPLC analysis with HPLC system Agilent Technologies model 1260 Infinity equipped with G1315D diode array detector. The mobile phase composed of acetonitrile and water (70:30). TEM image was taken and in vitro cytotoxic activity was studies against Sk-br-3 cell line.

RESULTS AND DISCUSSION
A colloidal dispersion of liposomes was prepared via thin film hydration method. Obtained liposomes was in size range of above 400 nanometer which then reduced to 30-120 nanometer by means of sonication method. The optimum liposomal formulation has zeta potential of +30 millivolt which is attributed to polar head group of phospholipids. The encapsulation efficiency of optimized formulation was 43% and 51% for paclitaxel and lapatinib, respectively. The release profile of optimized liposomal formulation showed minimum burst release at first two hours of release study (less than 5%) which was sustained to 53 and 64 hours for paclitaxel and lapatinib respectively.

Table 1: Tabulated formulation composition and Incorporation Efficiency

<table>
<thead>
<tr>
<th>Liposome composition</th>
<th>Drug/PL (mole %)</th>
<th>PTX/LPT (MR)</th>
<th>PTX I.E.</th>
<th>LPT I.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPC/Chol</td>
<td>11</td>
<td>2.3</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>Lecithin/Chol</td>
<td>11</td>
<td>2.3</td>
<td>43</td>
<td>51</td>
</tr>
<tr>
<td>DPPC/Lecithin/Chol</td>
<td>11</td>
<td>2.3</td>
<td>40</td>
<td>45</td>
</tr>
</tbody>
</table>

PTX: Paclitaxel, LPT: Lapatinib, MR: Molar Ratio, Cholesterol
I.E. = Incorporation Efficiency = drug incorporated in liposomes

TEM image of liposomal formulation showed spherical nanoparticles in range of 50 nanometer. In vitro cytotoxic study against Sk-br-3 cells showed superior toxic effect of liposomal formulation in comparison to each individual and also combination drugs as well.

CONCLUSION
This study presents a novel liposomal formulation capable of co-encapsulating paclitaxel and lapatinib, both are lipophilic agents, for overcoming paclitaxel associated Multi Drug Resistance. The liposomal formulation was prepared by thin film hydration method. In vitro cytotoxic studies against Sk-br-3 cell line showed increased toxic effect of this liposomal formulation in comparison to individual and combination drugs.

REFERENCES

Figure 1: SK-br-3 cytotoxic assay shows superior toxic effect of liposomal formulation in comparison drugs combination.

Figure 1: TEM image of liposomal formulation