Characterization of optimally designed liposomal formulation of Oxaliplatin

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ABSTRACT SUMMARY

PEG-coated liposomal Oxaliplatin (I-OHP) was designed to obtain further therapeutic benefit of I-OHP. The liposomal formulation of I-OHP showed an improved therapeutic effect and promising pharmaceutical profiles.

INTRODUCTION

I-OHP, an innovative third generation platinum compound, has powerful anti-neoplastic competence with no cross drug resistance with cisplatin and carboplatin (1). Although I-OHP is currently an essential therapeutic agent in treatment of advanced colorectal cancer, the non-selectivity between tumor site and normal tissue which is major cause of side effect poses a challenge for treatment of colorectal cancer using this compound.

Cancer chemotherapy by means of nanocarriers (e.g. PEG-coated liposome and polymeric micelles) has been believed to show desirable therapeutic efficacy and less side effect, because the nanocarriers selectively accumulate in tumor tissues due to enhanced permeability and retention (EPR) effect, which facilitates extravasation of nanocarriers from blood circulation into tumor tissue through gaps in the vasculature endothelium (2). Therefore, PEG-coated liposomal formulation of I-OHP (liposomal I-OHP) is expected to reduce non-selective distribution of the entrapped I-OHP and furthermore augment its accumulation within tumor tissues due to EPR effect, resulting in enhanced anti-tumor activity and reduced side-effect.

As mentioned above, our main purpose was to improve therapeutic efficacy of I-OHP by developing liposomal formulation of I-OHP. On the other side, simultaneous evaluation of the physicochemical properties is comparably important to develop the formulation as a medicinal product. Therefore, we also investigated \textit{in vitro} and \textit{in vivo} characteristics of liposomal I-OHP which we designed.

EXPERIMENTAL METHODS

The lipid composition of designed I-OHP liposomal formulation was the same as the formulation we previously reported (3). 5 mol\% of mPEG\textsubscript{2000}-DSPE to phospholipid was used as a PEG-lipid. The all lipids were dissolved with ethanol and then mixed with 8 mg/mL I-OHP solution using homogenizer. The resulting pre-liposome formulation was extruded through a polycarbonate membrane (400, 200 and 100 nm pore size) using an extruder device. Un-encapsulated, free I-OHP was removed by tangential flow filtration by means of ultrafiltration cassettes (PES, 300 kD). And, stabilizer and pH adjuster were added into the liposome formulation. Then the final liposome formulation was filtrated using 0.2 μm filter and further diluted for the animal injection. The particle size, particle shape, content of I-OHP and each lipid of the resulting liposome formulation were analyzed by dynamic light scattering method, cryo-TEM, HPLC, respectively. Also, the encapsulation efficiency was calculated using the analyzed data and revealed that the value is more than 99%. We can discuss preparation and analytical method in more detail.

For \textit{in vivo} antitumor study, HT-29 human colorectal tumor cells were transplanted subcutaneously into male nude mice. When tumor volume reached 100-200 mm\textsuperscript{3}, the animals were assigned randomly to control or drug treatment, and received drugs via tail vein. To assess antitumor effects, tumor volumes
were measured - using the formula \( L \times W^2/2 \), where \( L \) is the longest diameter and \( W \) is the shortest diameter of the tumor, and the tumor volumes were converted to values related to the initial tumor volume (relative tumor volume; RTV).

**RESULTS AND DISCUSSION**

The particle shape of liposomal 1-OHP was investigated by means of Cryo-TEM method (Fig. 1). Our liposome formulation showed relatively homogeneous particle size distribution (the DLS results showed same tendency and the vesicles with diameter mainly in the range from 50 to 150 nm are observed.) and the morphology was mainly, so-called single unilamellar vesicle. These properties were the similar feature as a commercially-available liposome formulation, Doxil/Caelyx.

![Fig.1, Cryo-TEM image of liposomal 1-OHP. The liposomal 1-OHP was homogeneous single unilamellar vesicle. Bar shows 100 nm.](image)

For assessing *in vivo* efficacy of liposomal 1-OHP, at first, plasma clearance and tumor accumulation of 1-OHP encapsulated in the formulation after i.v. administration were investigated. Liposomal 1-OHP showed, as expected, prolonged blood circulation time and efficient tumor accumulation of the entrapped 1-OHP (Data not shown in this abstract). Then, the anti-tumor activity of liposomal 1-OHP was evaluated in HT-29 tumor xenograft mouse model (Fig. 2). The liposomal 1-OHP formulation (4.2 and 7.0 mg/kg) showed significant tumor growth inhibition compared with conventional 1-OHP (7.0 mg/kg). Such efficient effect was dependent on the dose of 1-OHP. In addition, through this therapeutic experiment, no significant body weight loss was observed (data not shown). These results suggest that 1-OHP liposomal formulation improves anti-tumor activity of 1-OHP in HT-29 xenograft animal model without causing severe toxicity.

![Fig. 2, Anti-tumor effect of liposomal 1-OHP in HT-29 xenograft mouse model. Data represent mean ± S.D. (n=8). *, p<0.05 versus 1-OHP (7.0 mg/kg).](image)

**CONCLUSION**

The main purpose of this study was to investigate the characterization of the designed 1-OHP liposomal formulation. We could show that the formulation has not only favorable feature upon therapeutic (e.g. enhanced anti-tumor activities with no side-effect) but also promising profile as medicinal products.

**REFERENCES**