EFFICIENCY OF FOLATE-DECORATED METHOTREXATE NANOPARTICLES IN MCF-7

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Abstract - This study aim to prepare targeted nanoparticles from tri and di-block copolymer of PEGylatedP(CL) and investigate in vitro study in MCF-7. MTX conjugated copolymers at 10% and subsequently decorated with FOL-conjugated copolymers at 5 were prepared from PCL-PEG-PCL (tri-block pattern) and mPEG-PCL (di block pattern) via click reaction. After that, non-targeted and targeted nanoparticle were prepared by dialysis method. Four type of nanoparticles were investigated their characteristics and cytotoxicity. The results found that the targeted nanoparticles were toxic to MCF-7 than non-targeted nanoparticles. Moreover, targeted nanoparticles form tri-block pattern were effective than di-block nanoparticles due to the different formation of nanoparticles.

Keywords - Targeted nanoparticles, methotrexate, folic acid, MCF-7

I. INTRODUCTION

Recently, numerous studies evaluated the significance of nano particles for cancer drug delivery. Biodegradable polymers such as poly (caprolactone) (PCL) are widely investigated for the preparation of nanoparticles. Particularly, polymeric nanoparticles having amphiphillic block with poly ethylene glycol (PEG) such as PCL-PEG-PCL and PCL-mPEG have gained attention in controlled drug delivery. In these block copolymers PEG block forms the outer shell of nano particles whereas PCL due their hydrophobic nature forms nano particle core. PEG is well known for its non-antigenic and non immunogenic nature. In addition, because of its hydrophilic nature it facilitates the diffusion of water into nanoparticles matrix and provides diffusion mediated drug release from nanoparticles. PCL is hydrophobic biodegradable polyester which enables high permeability for small molecules .Being hydrophobic in nature it also provides good encapsulation efficiency to lipophilic drugs via hydrophobic interactions.PCL and PEG both tri-block copolymers (i.e. PCL-PEG-PCL) and di-block copolymer (mPEG-PCL)are widely explored for drug delivery. In attempt to sustain the drug release profile, these amphiphillic block copolymer were developed to conjugate with model molecules such as anticancer drug and targeting ligand.In this study, tri- and di-block copolymers of PEGylatedP(CL) conjugated with MTX and FOL were synthesized as previously reported (1). $P(MTXCLCL)_2$-PEG and $P(FOLCL)_2$-PEG were designated for tri-block pattern whereas P(MTXCLCL)-mPEG and P(FOLCL)-mPEG were designated for di-block pattern, respectively. Two architecture of nanoparticles, tri-block pattern and di-block pattern were prepared by dialysis method. The non-targeted nanoparticles and targeted nanoparticles and were characterized in term ofparticle size, polydispersity index, surface charge and % yield. Additionally, in vitro cytotoxicity of these nanoparticles were also investigated in MCF-7 cancer cell lines.

II. MATERIAL AND METHODS

A. Materials

Tri-bock copolymer ($P(MTXCLCL)_2$-PEG, $P(FOLCL)_2$-PEG) and di-block copolymers (P(MTXCLCL)-mPEG, P(FOLCL)-mPEG) were prepared as described somewhere (1).Methotrexate (MTX) was obtained from Suzhou Rovathin,Pharmatech Co., Ltd, China. Folic acid (FOL) was purchased from Fluka (Steinhheim, Germany). MCF-7 was obtained from NIHS, JCRB, Japan. All chemicals and solvents were analytical grade.

B.Preparation of nanoparticles

The non-targeted and targeted nanoparticles were prepared by dialysis. For tri-block pattern, non-targeted nanoparticles were prepared by drop $P(MTXCLCL)_2$-PEG solution in DMF into an aqueous volume of deionized water.After continuously stirring for an hour, the colloidal dispersion was enclosed in dialysis bag and dialyzed

against deionized water for 24 h. In case of targeted-nanoparticles, P(FOLCL)$_2$-PEG solution in DMSO were dropped into an aqueous dispersion of P(MTXCLCL)$_2$-PEG. For di-block pattern, non-targeted and targeted nanoparticles were prepared by the same method as described previously using P(MTXCLCL)-mPEG and P(FOLCL)-mPEG.

C. Nanoparticle characterization

1. Particle size and size distribution

The particle size (z-ave) and zeta potential of prepared nanoparticles was determined by ZetasizerNanoZS (Malvern Instrument Ltd., Malvern, UK) performed at 25 °C, 633 nm, and 173° angle. All results were the mean of three independent test runs.

2. Surface charge

The zeta potential (ZP) of nanoparticles was examined by ZetasizerNanoZS and calculated by the Helmholtz-Smoluchowskyequation obtained at 25 °C with a field strength of 20 V/cm.The data was obtained from the average of five measurements.

3. Yield of nanoparticles

The % yield of nanoparticles was calculated from an actual weight of lyophilized nanoparticles with respect to an initial weight of the polymers used for the nanoparticle preparation.

4.In vitro cytotoxicity

MCF-7 cells were plated at a density of 15×10^4 cells per well in MEM medium in 24-well plate and growth for 24 h. The cells were then exposed to a series of nanoparticles at different concentrations for 48 h. Then, viability of cells was measured using XTT assay. Effects of these nanoparticles on cell viability were calculated using untreated cells as control according to question (1). The half-maximal inhibitory concentration (IC_{50}) value was calculated by Hill equation , employing the KaleidaGraph^{TM} program (Version 3.08, Synergy Software, Reading, USA)

$$\% \text{ Viability} = \frac{(A_{490}\text{-}A_{650})_{\text{treated}}}{(A_{490}\text{-}A_{650})_{\text{control}}} \times 100 \qquad (1)$$

whereA_{490} and A_{650} are absorbance values at 490 and 650 nm, respectively.

IV. RESULTS AND DISCUSSION

1. Characterization of nanoparticles

The characteristics of tri- and di- block pattern of nanoparticles are presented in table 1. The particles size of tri-block pattern nanoparticles were larger than di-block pattern nanoparticles. Moreover, FOL-decorated nanoparticles(targeted nanoparticles) became larger than without FOL (non-targeted nanoparticles). This resultscan be explained by the formation of nanoparticles. Usually, nanoparticles prepared from such tri-block polymers have higher crystallinity of PCL block than di-block polymers. In addition, the larger of tri-block pattern nanoparticles was due to the hindrance of methotrexate molecule inside the core of nanoparticles.

In comparison, at the same pattern of nanoparticles, targeted nanoparticles were larger than non- targeted nanoparticles and more negative charge of zeta potential.This was resulted from the adsorption FOL molecule on the surface of nanoparticles as shown in Table 1. All formulations showed monodisperse size distribution with PI value less than 0.5. The yield of all nanoparticle preparation was half of initial weight. Moreover, the % yield of di-block pattern of nanoparticles was higher than that of tri-block pattern due to the more hydrophilic of mPEG in di-block polymer led to less aggregation during preparation of nanoparticles.

Formulations	z-ave (nm)	PI	ZP(mV)	Yield(%)
Tri-block pattern nanoparticles				
P(MTXCLCL)$_2$-PEG	132±1	0.283±0.048	-6.9±0.9	55.43±3.12
FOL-P(MTXCLCL)$_2$-PEG	266±10*	0.405±0.019	-12.4±0.9*	52.13±3.10
Di-block pattern nanoparticles				
P(MTXCLCL)-mPEG	101±1	0.117±0.001	-3.7±0.8	58.12±2.35
FOL-P(MTXCLCL)-mPEG	122±1*	0.180±0.008	-12.8±0.1*	56.98±1.23

Table 1 Particle size (z-ave), polydispersity index (PI), zeta potentials (ZP), and % yield of non-targeted and targeted nanoparticles

2. In vitro cytotoxicity

In vitro cytotoxicity of non-targeted and targeted nanoparticles was determined by XTT assay using and MCF-7 cells (positive folate receptor) as shown in table 2. The non-conjugated nanoparticles of tri- and di-block pattern of nanoparticles, P(MTXCLCL)$_2$-PEG and P(MTXCLCL)-mPEG had less toxicity than targeted nanoparticles. This result revealed the efficiency of MTX-conjugated polymer since free drug should be release from polymer backbone.In case of targeted nanoparticles (FOL-P(MTXCLCL)$_2$-PEG and FOL-P(MTXCLCL)-

mPEG) demonstrated more efficiency to kill MCF-7 cells as compared to the non-targeted nanoparticles. The toxicity of tri-block pattern nanoparticles, FOL-P(MTXCLCL)$_2$-PEG(IC_{50} = 0.18)was higher than FOL-P(MTXCLCL)-mPEG, di-block pattern nanoparticles (IC_{50} = 0.24).These results could be explained by various factor that have an effect on the cytotoxicity of nanoparticles such as PEG density, particle size and surface charge of polymeric nanoparticle including their biological properties.

Formulations	IC_{50} equiv. to MTX (µg/ml) MCF-7
Tri-block pattern nanoparticles	
P(MTXCLCL)$_2$-PEG	0.70
FOL-P(MTXCLCL)$_2$-PEG	0.18
Di-block pattern nanoparticles	
P(MTXCLCL)-mPEG	0.40
FOL-P(MTXCLCL)-mPEG	0.24

Table 2 IC_{50} values of FOL undecorated and decorated nanoparticles of tri-block copolymers and di-block copolymers in MCF-7 cells

CONCLUSION

MTX and FOL-conjugated block copolymer were successfully forming nanoparticles. Tri-block pattern nanoparticles copolymers had larger particle size than di-block pattern nanoparticles. For In vitro cytotoxicity, the targeted nanoparticles showed higher toxicity than non-targeted nanoparticles. This finding suggested the efficiency of MTX and FOL conjugated block copolymer as targeting drug delivery.

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