ABSTRACT SUMMARY

A novel polymeric micelle based on hyaluronic acid (HA) and phosphatidyl ethanolamines (PE) including Dipalmytoyl phosphatidyl ethanolamine (DPPE) and Distearoyl phosphatidyl ethanolamine (DSPE) were synthesized through conjugation reaction. HA-PE conjugates were confirmed by furrier transform-infrared (FT-IR) and ¹H nuclear magnetic resonance (¹H- NMR). Critical micelle concentration of HA-PE conjugates were analyzed and showed the lower CMC values for HA-DSPE. The solubilization efficiency of HA-PE micelles were analyzed for cholesterol as a hydrophobic model compound. Data showed that solubility of cholesterol was enhanced 300 folded relative to water by HA-PE micelle. Core-shell type structure of cholesterol loaded micelle was confirmed by SEM and HRTEM images. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) analysis showed conversion of crystalline cholesterol into amorphous state by entrapment in polymeric micelle. In vitro cytotoxic assay through breast cancer cells (MCF-7) showed no toxic effect of HA-PE polymers. Based on present study it can be concluded that polymeric micelle based on HA-PE conjugate is a promising vehicle for drug delivery of poorly soluble drugs not only for improving solubility of this system, but also because of biocompatibility of that.

INTRODUCTION

Polymeric micelle (PM) composed of di or triblock hydrophobic and hydrophilic polymers which formed self-assembled structures in aqueous media. These self-assembles have core-shell type structure in which hydrophilic fragments faced with water and formed shell part meanwhile the hydrophobic segments composed the core part of micelles due to hydrophobic interactions. The hydrophilic shell part of PMs made them unrecognizable for reticuloendothelial system during blood circulation since the core part of PMs made them stable¹. Hyaluronic acid (HA) is a natural polysaccharide which is made of repeating disaccharide units. HA is a biocompatible and biodegradable polymer which is derived from umbilical cord or roster combs. It has vast number of medical application and has been used regularly in drug delivery systems². Phospholipids are a class of lipids which contain phosphatidic acid group that classified as phospholipids. They composed of fatty acyl chains which made them hydrophobic compound. Due to their biocompatibility, phospholipids have been used interestingly in drug delivery systems³. Regarding to hydrophobic characterization and also biocompatibility of phospholipids, they could be considered as an attractive candidates for core part of PMs. In this research a novel polymeric micelle based on HA and different phosphatidyl ethanolamines (PE) were synthetized and evaluated for their physicochemical characteristics which could be served as a biocompatible drug carrier in pharmaceutical researches.

EXPERIMENTAL METHODS

HA-PE conjugated were synthetized by activation of carboxylic acid (-COOH) group of HA by 2 molar excess of EDC and NHS. The activated HA was reacted with 2 molar excess of either DPPE or DSPE for 24h. The resultant mixture was dialyzed through water for 48h followed by lyophilization. The HA-PE conjugates were characterized by 1H NMR and FTIR. Critical micelle concentration was measured spectrophotometrically with pyrene as a florescence probe at excitation wave length of 384 nm. For evaluation of solubilization efficiency of HA-PE micelle, cholesterol, a hydrophobic natural compound was selected. 3 mg of cholesterol was dissolved in 3 ml of methanol and added dropwise to solution of 1 mg/ml of either HA-DPPE or HA-DSPE. The mixture was sonicated in 50 °C for 10 min and stirred for further 1 hour at room temperature. The dispersion was centrifuged for 20
min at 4000 rpm followed by lyophilization. The amount of loaded cholesterol in micelle was determined spectrophotometrically at 205 nm. Solubilization efficiency (SE %) was determined by the ratio of loaded cholesterol in micelle to feeding amount of cholesterol during each formulation. Size distribution and zeta potential of micelle was analyzed by dynamic light scattering (DLS) method at room temperature. Physical characteristic of cholesterol in micelle was evaluated by differential scanning calorimetry (DSC) and X-ray diffraction (XRD) method. For morphological evaluation of micelles, scanning electron microscopy (SEM) and thermal electron microscopy (TEM) were performed. In order to investigate the toxicity effect of HA-PE conjugates, breast cancer cell line (MCF 7) was selected for in vitro cytotoxicity study using (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were cultured in DMEM culture medium containing 10% fetal bovine serum and 100 IU/mL penicillin and 100 mg/mL streptomycin at 37°C in 5% CO₂ atmosphere. The cytotoxicity of HA-PE conjugates were evaluated with sterile water as a blank. The relative cell viability (%) was calculated as (OD of treated cells with HA-PE conjugates/OD of treated cells with sterile water) × 100.

RESULTS AND DISCUSSION

HA-PE conjugates were synthetized successfully by coupling the –NH₂ group of PE to –COOH group of HA. Degree of substitution (DS %) was calculated 8.2% and 11.5% for HA-DSPE and HA-DPPE, respectively. The CMC value for HA-DSPE and HA-DPPE were 29.8 and 73.8 µg/ml. SE% of cholesterol for HA-DSPE and HA-DPPE micelle were calculated 31.4 and 27.2 µg/ml, respectively which shows 300 fold increasing in solubility of cholesterol relative to water. The hydrodynamic particle size was in range of 90 nm and zeta potential was about -27.9 mV. Thermal analysis represented the amorphous state of cholesterol in micelle which is confirmed by XRD result. The SEM and TEM images showed the micelle was spherical in shape with core-shell like structure. Cytotoxicity studies showed no evidence of toxic effect of HA-PE polymer through MCF 7 cell line.

CONCLUSION

Different types of novel polymeric micelle based on HA-PE amphiphilic graft copolymer were developed for encapsulation of cholesterol. These novel micelles represented core-shell type structure with a small particle size and excellent solubilization efficiency of cholesterol. Based on present study it can be concluded that HA-PE micelle is a suitable delivery system for hydrophobic drugs not only for their high solubilizing effect but also for their biocompatibility and biodegradability.

References

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