Abstract

Triple Negative Breast Cancer (TNBC), is an extremely heterogeneous disease lacking of estrogen receptor (ER), progesterone receptor (PR), as well as human epidermal growth factor receptor-2 (HER-2). Absence of these receptors caused ineffectiveness of chemotherapy to TNBC. At present, adjuvant chemotherapy is the only available treatment for TNBC. However, the TNBC may express other proteins which can be targeted by using specific antibodies. The cytokeratin 5/6 (CK5/6) expression is noted to be a potential biomarker that is highly associated with TNBC patients among Malaysian woman. The present study was aimed at development of Lapatinib loaded nanoparticles (LPT-NPs) anchored with CK5/6 monoclonal antibody (mAb) for the treatment of TNBC. The PEGylated PLGA-LPT-NPs were formulated by nanoprecipitation method and subsequently covalently conjugated with CK5/6 mAb *via* cross linking to form the immunonanoparticles (INPs). The NPs were characterized by morphology, particle size, zeta potential, entrapment efficiency and drug release study. The LPT-NPs exhibit spherical shape with smooth surface, whereas the INPs were coated with a thin layer of CK5/6 mAb. The particle size of the NPs was 316.4 nm (LPT-NPs) and 338.5 nm (INPs) respectively. PDI values of 0.4 and 0.3 with surface charge of -13.5 mV and -4.2 mV were noted for LPT-NP and INP, respectively. Drug entrapment efficiency of 78.2 ± 2.1 % and 80.2 ± 1.5 % was noted for LPT-NPs and INPs respectively. Both NPs demonstrated a biphasic drug release pattern comprising of an initial burst release (15.4 ± 1.4 to 17.8 ± 0.5 %), followed by sustainable release (78.3 ± 2.4 % to 83.3 ± 1.3 %). The conjugated mAb remained intact and similar was determined by SDS-PAGE electrophoresis and quantified by Bradford protein assay. About 20.3 µg of CK5/6 mAb was quantified in 100 milligram of NP formulations. Cytotoxicity of the INPs was examined on MDA-MB-231 breast cancer cell lines by MTT assay. The percentage cell viability significantly reduced to 20.2 ± 1.6 % and 10.6 ± 3.4 % after treated with LPT-NPs and INPs at 48 hours, respectively. Cell uptake study of NPs was confirmed by fluorescence microscopy using FITC as marker. Western blot method confirmed the expression of CK5/6 protein in the MDA-MB-231 cells. The cell expressing CK5/6 protein was amplified by PCR and subjected to Agarose gel electrophoresis. Flowcytometric analysis of the INPs exhibited greater apoptotic potential against cancer cells compared to LPT-NPs. The study provides an insight for further investigation of the developed PEGylated PLGA-LPT-CK5/6 INPs as a potential therapeutic strategy for TNBC treatment.

Keywords: Triple Negative Breast Cancer, CK5/6 protein, Lapatinib, PEGylated-PLGA