ABSTRACT

PRIMARY BREAST CANCER CELLS DEATH INDUCED BY 4-(*t*-BUTYL)-*N*-BENZOYLUREA: IN VITRO MECHANISM

Aguslina Kirtishanti

EGFR and HER2 positive breast cancer patients demonstrate poor prognosis in medical history. The over expressions of EGFR and HER2 enhance Ras-MAPK and JAK-STAT3 signaling pathways activation, leading to the increasing proliferation of breast cancer cells. The limited therapeutic options and drug resistance encourage further studies to obtain anticancer drugs with mechanisms for breast cancer cell death through analysis of inhibiting EGFR and HER2 and increased ROS. The cytotoxicity of 4-(*t*-butyl)-*N*-benzoylurea (4TBBU) compound was confirmed by the Brine Shrimp Lethality Test and it has the potential to be investigated further its molecular mechanism.

This present experiment was performed by resynthesizing 4TBBU compound. Undergoing the purity test, the synthesized compound structure was confirmed with IR, ^{1}H-NMR, ^{13}C-NMR, and HRMS spectroscopy. The cancer cells used are primary breast cancer cells isolated from the tumor tissue of breast cancer patient that express EGFR and HER2. Cytotoxic activity test was performed under MTT assay and analysis of its molecular mechanism using immunofluorescence and flow cytometry assays. The molecular mechanism analysis examined the expressions of pEGFR, pHER2, pRas, pSTAT3, Ki67 proteins, MDA metabolites, ROS, necrosis, and apoptosis.

Based on the cytotoxic test, 4TBBU compound has higher cytotoxicity than hydroxyurea, yet lower compared to lapatinib. It also indicates great selectivity against breast cancer primary cells. The analysis of the molecular mechanism suggests that 4TBBU compound acts on lowering the expressions of pEGFR, pHER2, pRas, pSTAT3, Ki67, enhancing MDA metabolites, and inducing necrosis in primary breast cancer cells. However, 4TBBU has not been able to increase ROS and apoptosis. Based on the path analysis, 4TBBU compound influenced the decrease in pEGFR expression then affect the decrease in Ki67 expression directly without going through Ras and STAT3 pathway.

This study concludes that 4TBBU compound could inhibit EGFR signaling which directly affects a decrease in Ki67 expression without going through Ras and STAT3 pathways and could induce necrosis in primary breast cancer cells.

Keywords: 4-(*t*-butyl)-*N*-benzoylurea, cell death mechanism, primary breast cancer cells, in vitro, EGFR, HER2, ROS.