ABSTRAK

Latar Belakang: Kalkon-3 bersifat sitotoksik dan selektif terhadap kanker payudara subtipe luminal, yang diduga terjadi melalui mekanisme hambatan aktivasi *epidermal growth factor receptor* (EGFR). Aktivitas antikanker kalkon-3 belum pernah diteliti secara *in vitro* baik pada subtipe luminal, TNBC, maupun *in vivo* pada jaringan kanker payudara tikus yang diinduksi DMBA.

Tujuan: Mengetahui efek sitotoksik dan aktivitas antikanker kalkon-3 pada *downstream target* EGFR sebagai agen antiproliferasi, proapoptosis, antiangiogenesis, dan antiinvasif pada sel kanker payudara manusia galur T-47D, MCF -7, MDA-MB-231 dan kanker payudara tikus yang diinduksi DMBA.

Metode: Sel T-47D, MCF-7, MDA-MB-231 diinkubasi dengan kalkon-3 serial konsentrasi. IC_{50} dan hambatan proliferasi dianalisis menggunakan uji MTT. Distribusi populasi siklus sel, distribusi sel apoptosis dan nekrosis, ekspresi pEGFR, cyclin-D1, caspase-9, Bcl-xL, Bak, VEGF dan E-cadherin dianalisis dengan *flow cytometry*. Migrasi sel dievaluasi dengan *scratch wound healing assay* pada interval waktu tertentu. Tikus DMBA dengan nodul tumor diberi perlakuan dengan kalkon-3 (25, 50, atau 100 mg/kg) selama 3 minggu dan dipalpasi setiap minggu untuk menentukan ukuran, jumlah, dan lokasi tumor. Aktivitas antikanker kalkon-3 dikaji berdasarkan multiplisitas, ukuran tumor, tipe histologis, derajat histologis, dan ekspresi protein EGFR, pEGFR, cyclin-D1, caspase-3, caspase-9, Bcl-xL, dan VEGF .

Hasil: Kalkon-3 bersifat sitotoksik dan selektif terhadap sel T-47D, MCF-7 dan MDA-MB-231, dengan mekanisme antiproliferatif melalui hambatan siklus sel pada fase G1, S, dan G2/M, yang dimediasi oleh cyclin-D1. Kalkon-3 menginduksi apoptosis melalui jalur intrinsik yang diinisiasi oleh caspase-9 dan menurunkan ekspresi Bcl-xL. Kalkon-3 menghambat angiogenesis melalui hambatan ekspresi VEGF. Kalkon-3 menghambat migrasi dan invasi sel kanker payudara MDA-MB-231 dengan meningkatkan ekspresi E-cadherin. Aktivitas antikanker kalkon-3 diperantarai oleh hambatan aktivasi EGFR. Selaras dengan hasil *in vitro*, aktivitas antikanker kalkon-3 pada kanker payudara tikus yang diinduksi DMBA terjadi melalui hambatan aktivasi EGFR, hambatan ekspresi cyclin-D1, dan induksi apoptosis yang ditandai dengan peningkatan ekspresi caspase-3 aktif melalui jalur intrinsik yang diinisiasi caspase-9, serta hambatan angiogenesis dengan menghambat ekspresi VEGF.

Kesimpulan: Kalkon-3 berpotensi sebagai obat anti kanker untuk mengobati kanker payudara.

Kata kunci: Kalkon-3, aktivitas antikanker, kanker payudara

ABSTRACT

Background: Chalcone-3 has been shown to be cytotoxic and selective against human luminal subtype breast cancer cell lines, which are suspected to occur through the mechanism of epidermal growth factor receptors (EGFR) inhibition. Anti-cancer activity of chalcone-3 has never been studied *in vitro* in both luminal and TNBC subtype, as well as *in vivo* in rat breast cancer tissue induced by DMBA.

Objective: To identify the cytotoxic effect and anticancer activity of chalcone-3 in the downstream targets of EGFR as an antiproliferative, proapoptosis, antiangiogenesis, and antiinvasive agents in human breast cancer T-47D, MCF-7, MDA-MB-231 cell lines and breast cancer in DMBA-induced rat.

Methods: T-47D, MCF-7, MDA-MB-231 cells were incubated with serial concentration of chalcone-3. The IC_{50} and proliferation inhibition were analyzed using MTT assay. Distribution of cell cycle population, apoptotic and necrotic cells, pEGFR, cyclin-D1, caspase-9, Bcl-x_L, Bak, VEGF and E-cadherin expression were analyzed using flow cytometry. Cell migration was evaluated by a scratch wound healing assay at specified time intervals. DMBA-induced rat bearing tumor were given chalcone-3 (25, 50, or 100 mg/kg) for 3 weeks and palpated weekly to determine the size, number, and location of tumors. The anti-cancer activity of chalcone-3 was studied based on multiplicity, tumor size, histological type, histological grade, and protein expression of EGFR, pEGFR, cyclin-D1, caspase-3, caspase-9, Bcl-xL, and VEGF.

Results: Chalcone-3 is cytotoxic and selective against T-47D, MCF-7 and MDA-MB-231 cells, with an anti-proliferative mechanism through cell cycle arrest in G1, S, and G2/M, which is mediated by cyclin-D1. Chalcone-3 induced apoptosis through the intrinsic pathway initiated by caspase-9 and decreased Bcl-xL expression. Chalcone-3 inhibits angiogenesis through inhibition of VEGF expression. Chalcone-3 inhibits migration and invasion of MDA-MB-231 breast cancer cells by increasing E-cadherin expression. The anti-cancer activity of chalcone-3 is mediated by inhibition of EGFR activation. In line with in vitro results, chalcone-3 has anti-cancer activity in breast cancer of DMBA-induced rat, by inhibiting EGFR activation, cyclin-D1 expression, and inducing apoptosis which is indicated by the increased expression of active caspase-3 through the intrinsic pathway initiated by caspase-9, as well as inhibits angiogenesis by inhibiting VEGF expression.

Conclusion: Chalcone-3 has the potential as an anti-cancer drug to treat breast cancer.

Keywords: Chalcone-3, anticancer activity, breast cancer