

ABSTRACT

The combination chemotherapy of doxorubicin (DOX) and cyclophosphamide (CYC) (AC) is the most commonly used regime for treating breast cancer, but it can cause premature ovary failure and infertility in premenopausal breast cancer survivors. AC is cytotoxic to tumor cells and each causes DNA fragmentation, ROS generation and apoptosis. Tocopherols are antioxidants which can reduce the damage caused by ROS generation. **The objectives:** to examine if DOX (5, 10 and 25 μ M), HCYC (0.5, 1 and 2.5 μ M), alpha tocopherol (A-TOC) (50, 75 and 100 μ M) or gamma tocopherol (G-TOC) (50, 75 and 100 μ M) alone will cause dose-dependent cytotoxicity in MCF-7 breast cancer cells and KGN granulosa cells; if the combination of AC or AC plus tocopherols will be more cytotoxic than either chemotherapeutic alone; if the EC₂₅, EC₅₀ and EC₇₅ values of DOX, HCYC, A-TOC and G-TOC cause a dose-dependent increase in apoptosis, and if the combination of EC₂₅ value of DOX and EC₂₅ value of HCYC will be cytotoxic more than 50% of MCF-7 cells and if the combination of EC₂₅ values of DOX, EC₂₅ value of HCYC and A-TOC or G-TOC will be cytotoxic more than 75% of MCF-7 cells. **The methods:** applied the crystal violet (CV) and MTT cytotoxicity assays to examine different doses of DOX, HCYC, A-TOC and G-TOC and their combinations at four different exposure periods which were 24hr exposure, followed by 24hr or 48hr culture, and 72hr continuous exposure, in MCF-7 and KGN cell lines; use the CV assay to determine the EC₂₅, EC₅₀ and EC₇₅ values of DOX, HCYC, A-TOC and G-TOC after 24hr exposure or 24hr exposure with 24hr culture in MCF-7 cells; use Annexin V and PI staining to examine MCF-7 cells apoptosis after exposure to different doses of chemotherapeutics and tocopherols, and their combinations corresponding to their EC₂₅, EC₅₀ and EC₇₅ values. **The result:** in both the CV and MTT assays all tested concentrations of HCYC and A-TOC were not cytotoxic, but DOX (5, 10 and 25 μ M) was significantly cytotoxic to MCF-7 and KGN cells. G-TOC was cytotoxic to MCF-7 cells but not to KGN cells. The combinations of AC or AC with tocopherols did not have synergistic cytotoxicity

towards MCF-7 and KGN cells except when a lower initial seeding density (10,000 cells/well) was used. In these conditions the combination of AC had a synergism effect in the MTT assay after 24hr exposure to MCF-7 cells, and the combination of AC or the combination of AC with alpha or gamma tocopherols had synergistic cytotoxicity to KGN cells. The EC50 value of DOX was 3.63uM after 24hr exposure and 3.24uM after 24hr exposure with 24hr culture. The EC50 value of HCYC was 63.69uM after 24hr exposure and 54.65uM after 24hr exposure with 24hr culture. The EC50 value of G-TOC was 105.3uM after 24hr exposure and 78.13uM after 24hr exposure with 24hr culture. The EC50 value could not be determined for A-TOC as the highest concentration tested, 500uM, was still not cytotoxic to MCF-7 cells. DOX, HCYC, A-TOC and G-TOC and their combinations did not cause MCF-7 cell apoptosis. In the single experiment replicate that could be complicated in the time available.