

Citral induced apoptosis in MDA-MB-231 spheroid cells

ABSTRACT

Background: Breast cancer remains a leading cause of death in women worldwide. Although breast cancer therapies have greatly advanced in recent years, many patients still develop tumour recurrence and metastasis, and eventually succumb to the disease due to chemoresistance. Citral has been reported to show cytotoxic effect on various cancer cell lines. However, the potential of citral to specifically target the drug resistant breast cancer cells has not yet been tested, which was the focus of our current study.

Methods: The cytotoxic activity of citral was first tested on MDA-MB-231 cells in vitro by MTT assay. Subsequently, spheroids of MDA-MB-231 breast cancer cells were developed and treated with citral at different concentrations. Doxorubicin, cisplatin and tamoxifen were used as positive controls to evaluate the drug resistance phenotype of MDA-MB-231 spheroids. In addition, apoptosis study was performed using AnnexinV/7AAD flowcytometry. Aldefluor assay was also carried out to examine whether citral could inhibit the ALDH-positive population, while the potential mechanism of the effect of citral was carried out by using quantitative real time- PCR followed by western blotting analysis.

Results: Citral was able to inhibit the growth of the MDA-MB-231 spheroids when compared to a monolayer culture of MDA-MB-231 cells at a lower IC₅₀ value. To confirm the inhibition of spheroid self-renewal capacity, the primary spheroids were then cultured to additional passages in the absence of citral. A significant reduction in the number of secondary spheroids were formed, suggesting the reduction of self-renewal capacity of these aldehyde dehydrogenase positive (ALDH+) drug resistant spheroids. Moreover, the AnnexinV/7AAD results demonstrated that citral induced both early and late apoptotic changes in a dose-dependent manner compared to the vehicle control. Furthermore, citral treated spheroids showed lower cell renewal capacity compared to the vehicle control spheroids in the mammosphere formation assay. Gene expression studies using quantitative real time PCR and Western blotting assays showed that citral was able to suppress the self-renewal capacity of spheroids and downregulate the Wnt/ β -catenin pathway.

Conclusion: The results suggest that citral could be a potential new agent which can eliminate drug-resistant breast cancer cells in a spheroid model via inducing apoptosis.

Keyword: Citral; Wnt; Spheroids; ALDH1