Inhibiting Fatty Acid Amide Hydrolase (FAAH) Induces Apoptosis in Breast Cancer Cells

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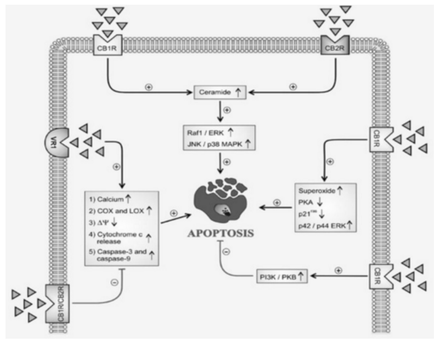
Abstract

Background: Breast cancer is a highly heterogeneous, aggressive disease that has poor prognosis. Current treatment is present, but research on a therapeutic alternative is ongoing. Endocannabinoids, members of the endocannabinoid system, are naturally produced by cells in our bodies. They are diverse signaling components that have several functions, including regulation of the apoptosis mechanism. Fatty Acid Amide Hydrolase (FAAH) is an integral membrane enzyme that hydrolyzes endocannabinoids, rendering them inactive. Since FAAH is upregulated in cancer cells, FAAH inhibition is predicted to increase the apoptotic rate.

Methods: To test the effects of FAAH inhibitors and exogenous endocannabinoids, cells were cultured, probed for FAAH expression using Western blot analysis, treated with FAAH inhibitors, exogenous endocannabinoids, and combinations of both, all in varying doses, and cell viability was measured using MTT assays.

Results: High levels of FAAH were observed in two breast cancer cell lines. The greatest change was observed at 50 µm PF-750 treatment, which is the most selective FAAH inhibitor. URB597 combination treatments decreased cell viability in a supra-additive manner. The most significant change was observed at co-treatment levels of 50 µm URB597 and 50 µm AEA, which resulted in a 30% decrease ($p<0.001$) in cell viability compared to vehicle control. FAAH inhibition was more effective than exogenous endocannabinoid use, and combination treatments of FAAH inhibitors and exogenous endocannabinoids, both at the highest doses, were the most effective.



Binding of extracellular anandamide (triangles) to type 1 or 2 cannabinoid receptors(CB1R or CB2R) triggers different signal transduction pathways, depending on the cell type. Activation of either CB1R or CB2R increases intracellular levels of ceramide, which activates Raf1/ERK cascade, thus engaging JNK/p38 MAPK along the pathway leading to apoptosis.

Study Design

Establish protocol

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Probe for FAAH Expression

↓

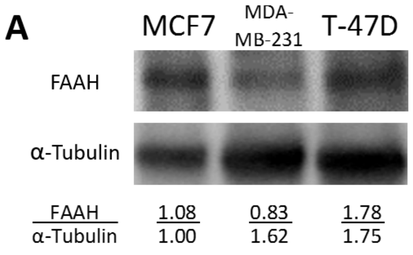
Grow cells and add FAAH inhibitor/endocannabinoid treatment

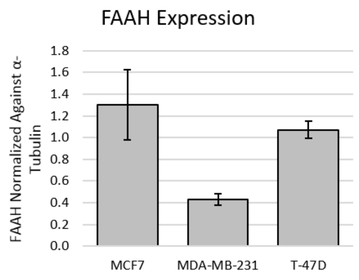
↓

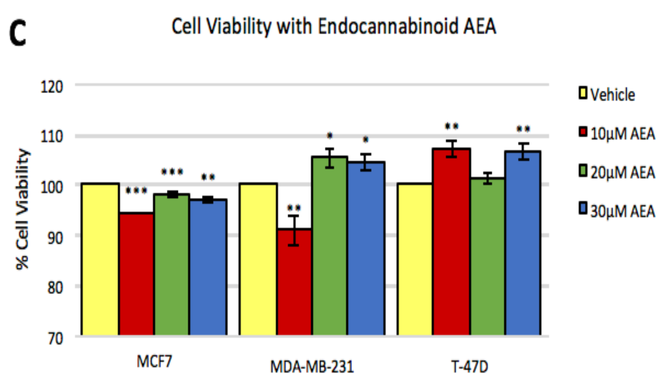
Measure cell viability

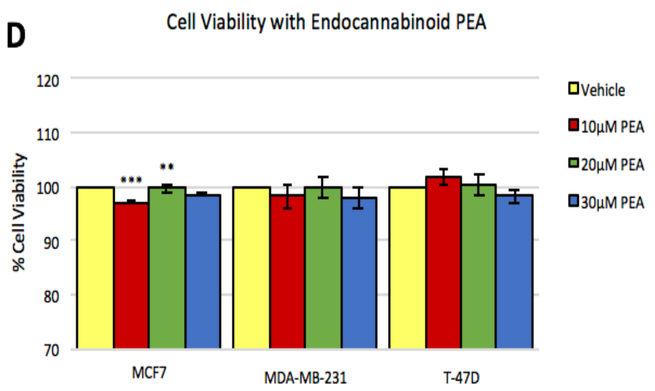
Western blot analysis was used to probe for FAAH expression in the human breast cancer cell lines. Then, individual apoptotic effects of FAAH inhibitors (URB597 and PF750) and endocannabinoids (AEA and PEA) were tested using MTT assays.

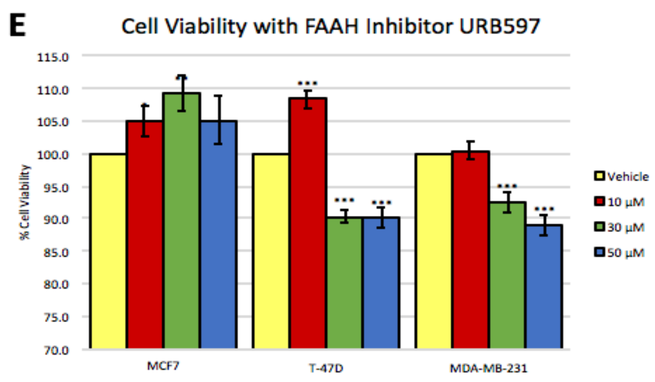
Study Results Part 1







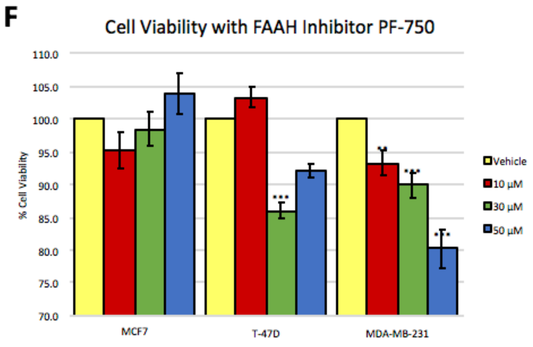


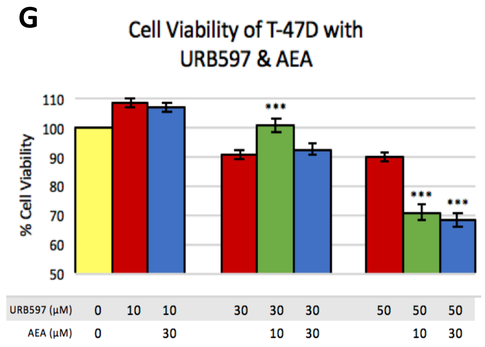


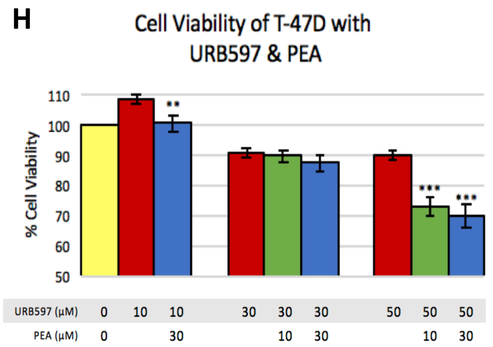
(A) Western Blot demonstrating expression of FAAH, using α-Tubulin as house-keeping control. (B) FAAH shown normalized against α-Tubulin. (C) Cell viability (%) of breast cancer cell lines MCF7, MDA-MB-231, and T47D, caused by endocannabinoid, AEA. (D) Cell viability (%) of breast cancer cell lines MCF7, MDA-MB-231, and T47D, caused by endocannabinoid, PEA. (E) Cell viability (%) of breast cancer cell lines MCF7, MDA-MB-231, and T47D, caused by FAAH inhibitor, URB597. Data are expressed as percent cell viability normalized to the vehicle control with n≥3. Statistical significance are relative to vehicle control and are assigned as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Conclusion: FAAH inhibitor, URB597, produces varying effects in different cell lines, but is more effective than exogenous endocannabinoids, AEA and PEA, and has more of a dose-dependent response for cell viability.

Study Results Part 2







(F) Cell viability (%) of breast cancer cell lines MCF7, MDA-MB-231, and T47D, caused by FAAH inhibitor, PF-750. (G) Cell viability (%) of breast cancer cell lines MCF7, MDA-MB-231, and T47D, caused by combination treatment of FAAH inhibitor, URB597, and endocannabinoid, AEA. (H) Cell viability (%) of breast cancer cell lines MCF7, MDA-MB-231, and T47D, caused by combination treatment of FAAH inhibitor, URB597, and endocannabinoid, PEA. Data are expressed as percent cell viability normalized to the vehicle control with n≥3. Statistical significance are relative to vehicle control and are assigned as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Conclusion: FAAH inhibitor, PF-750, produced the most promising results in cell line, MDA-MB-231, as did URB597 for this cell line. FAAH inhibitor and exogenous endocannabinoid combination treatment is the most effective means of decreasing cancer cell viability, especially at the highest doses of each.

Conclusions

FAAH inhibition is a promising approach to induce cell death in breast cancer cells. Studies are in progress to test the in-vivo efficacy of FAAH inhibition and the underlying pathogenic mechanisms.

References

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