

Exploring the Potential Future Uses of Palmitoylethanolamide for the Treatment of Cancer.

Contents Page

Introduction	3
Palmitoylethanolamide's Intrinsic Anti-cancer Effects	4
Anti-proliferative effects	4
Inducing Cell Cycle Arrest	7
Reducing Tumour Cell Migration	9
Potential Applications of Palmitoylethanolamide in the Medical Field	11
Palmitoylethanolamide in Chemotherapy	11
Palmitoylethanolamide in Radiotherapy	14
Conclusion	16
References	17

Introduction

Palmitoylethanolamide (PEA) is a bioactive lipid mediator belonging to the N-acyl-ethanolamine (NAE) fatty acid amide family (Clayton et al., 2021). First identified inside egg yolk, soybean, and peanut oil in 1957, PEA is known for its anti-inflammatory and analgesic properties (Rankin and Fowler, 2020). The fatty acid is an endogenous compound (Rankin and Fowler, 2020), which means it is produced in every tissue of the body, including the brain (Clayton et al., 2021). PEA is synthesised within the phospholipid bilayer and therefore acts locally (Clayton et al., 2021).

In the form of Levagen® and Levagen®+, a high-quality PEA and cold water dispersible respectively, PEA is used to relieve acute joint pain, dissipate headaches, increase exercise performance and benefits, support restful sleep, and promote immune health (Gencor, 2023a)(Gencor, 2023b). Clinical trials have further suggested PEA's potential benefit with a greater range of afflictions such as rheumatic arthritis, chronic pain, atopic eczema (Rankin and Fowler, 2020), and cancer.

This report will focus on PEA's potential for cancer treatment and examine possible uses for the chemical in the field of oncology.

Palmitoylethanolamide's Intrinsic Anti-cancer Effects

This section will address the characteristics of PEA that make it intrinsically effective in cancer treatment, specifically focusing on the chemical's anti-proliferative effects, ability to induce cell cycle arrest, and its role in reducing tumour cell migration.

Anti-proliferative effects

PEA is known to act in synergy with arachidonylethanolamide (AEA), an endogenous ligand for the cannabinoid type 1 and type 2 receptors (Di Marzo et al., 2001). AEA has been shown to exhibit anti-proliferative effects on human breast cancer cells, which is enhanced significantly by PEA (Di Marzo et al., 2001). This is partially due to the fact that PEA inhibits the expression of fatty acid amide hydrolase (FAAH), a hydrolytic enzyme involved in catalysing AEA degradation (Di Marzo et al., 2001)(Rankin and Fowler, 2020).

Figure 1.1 - Graph depicting the dose-dependent potentiation by PEA of the cytostatic effect of AEA in two different human breast cancer cell lines (Di Marzo et al., 2001)

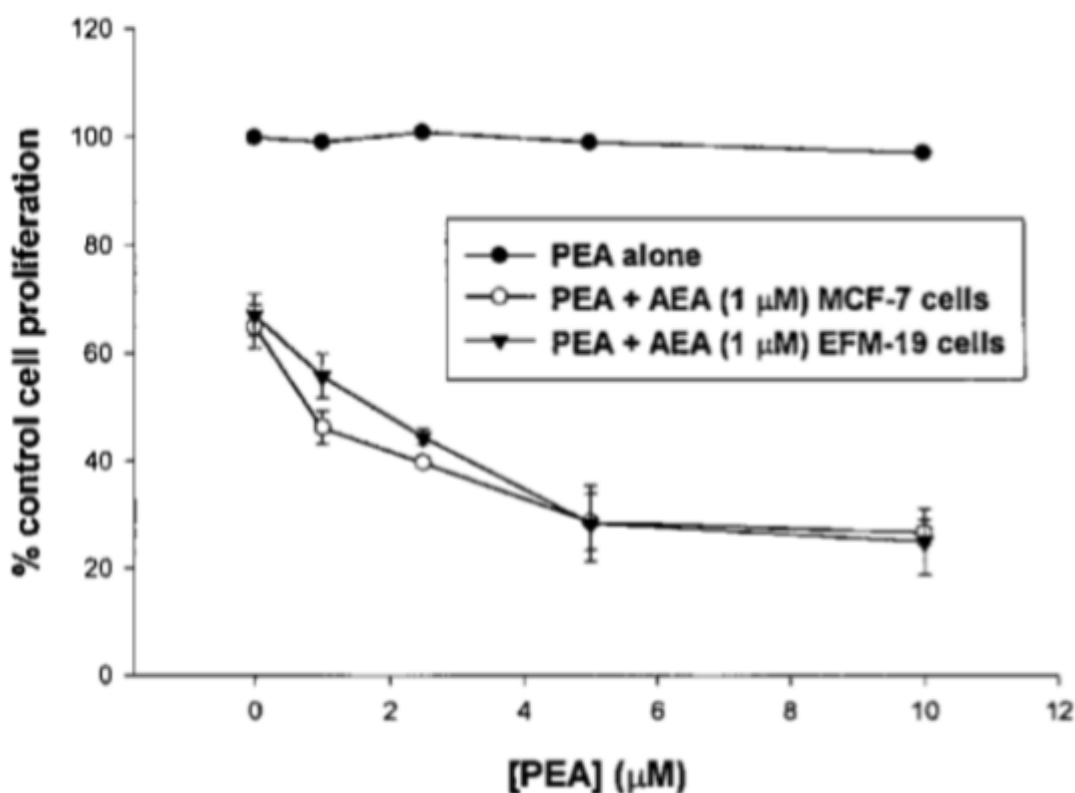


Figure 1.1 demonstrates how when acting alone, PEA has little to no effect on cancer cell lines, as seen with the near straight line at 100% proliferation for PEA alone. This could be because the anti-proliferative effects associated with AEA are credited to interactions with either type 1 cannabinoid receptors or vanilloid-like receptors, to which PEA has little affinity (De Petrocellis et al., 2002). However, Figure 1.1 supports the notion that AEA is the chemical responsible for the anti-proliferation effect, as once added with PEA there is a decrease in the cell proliferation of two varying human breast cancer cell lines. The error bars for both breast cancer cell lines overlap, implying little difference between the effects of AEA on different types of breast cancer, but there is no overlap with PEA alone suggesting statistical significance in the decrease in percentage proliferation observed.

Figure 1.2 - Graph displaying the effect of 5 μ M PEA on the dose-dependent inhibition of HBCC proliferation by AEA. (Di Marzo et al., 2001)

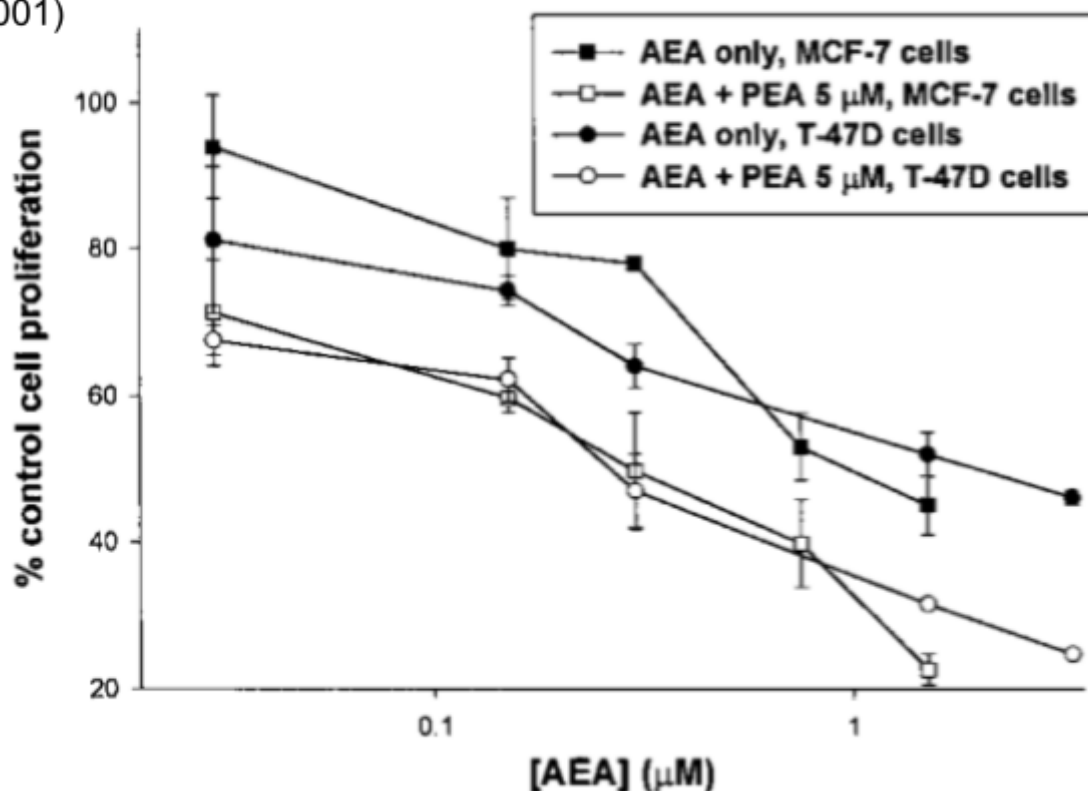


Figure 1.2 depicts how PEA can enhance the anti-proliferative effects of AEA. For both human breast cancer cell lines, AEA only led to a decrease in percentage proliferation as AEA concentration increased, however with the addition of 5 μ M of PEA, both cell lines saw a downward shift in proliferation. This shows how PEA can act as an ‘entourage’ compound for AEA, functioning as an endogenous enhancer of AEA biological actions (De Petrocellis et al., 2002).

The data of both figures came from the same source thus the same procedure was carried out for each human breast cancer cell line’s cell proliferation assays (Di Marzo et al., 2001), making the results more reliable. However, the T-47D and MCF-7 cells were purchased from A.T.C.C. while EFM-19 cells were purchased from DSM (Di Marzo et al., 2001). Since the paper mentions culturing cells “as advised by the manufacturers” (Di Marzo et al., 2001), this makes Figure 1.1 less reliable as the cell lines originated from different manufacturers and could have had slightly different culturing procedures.

Inducing Cell Cycle Arrest

Ultramicronized palmitoylethanolamide (um-PEA) promotes cell cycle arrest in colorectal cancer cells (Pagano et al., 2021). This arrest occurs in the second growth phase and mitosis (G2/M) and could potentially be due to the increased expression of the cyclin B1 and CDK1 complexes which is the main executor of the G2/M phase (Pagano et al., 2021). Therefore, the sustained expression of these regulators may be inducing cell cycle arrest.

Figure 2.1 - Graph indicating the cell cycle analysis of HCT116 cells treated or not with um-PEA by flow cytometry (Pagano et al., 2021)

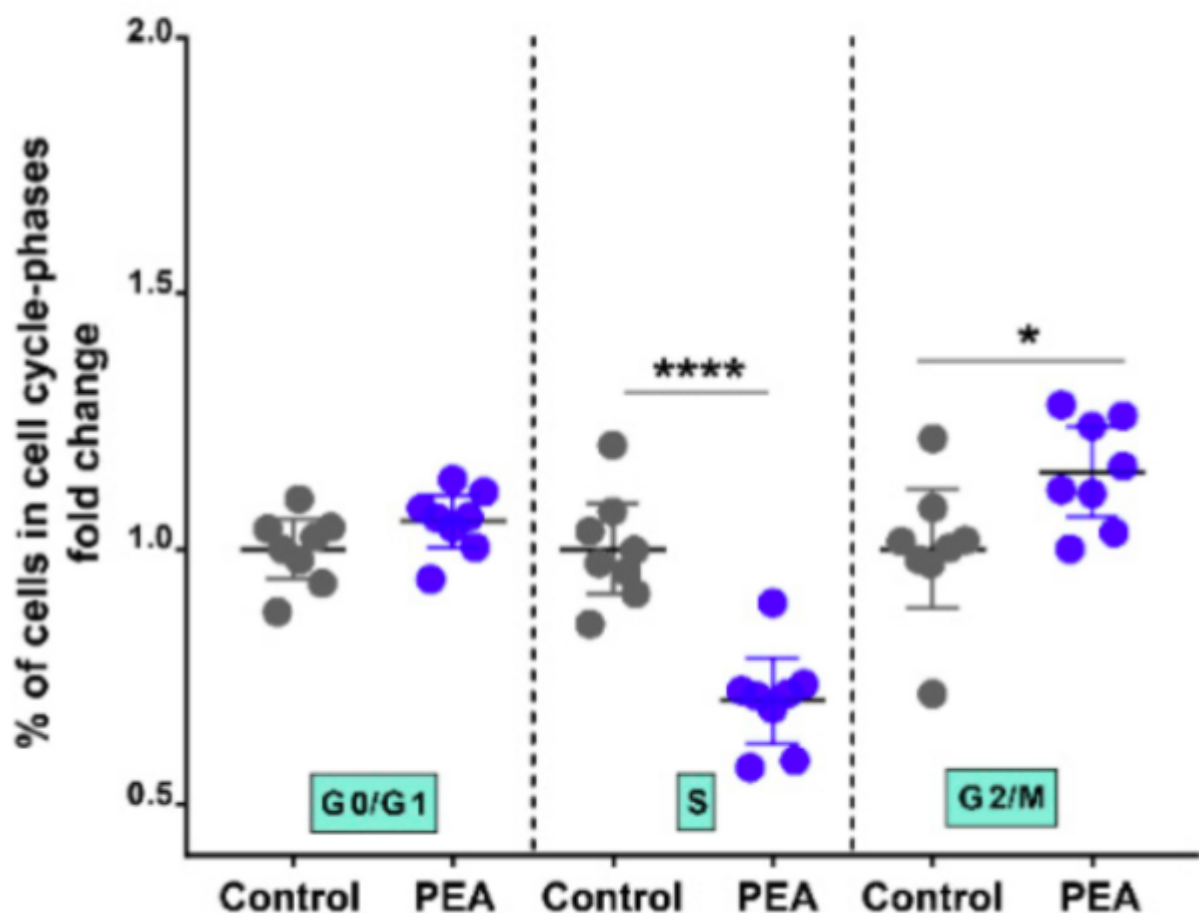


Figure 2.1 depicts a greater percentage of cells in the G2/M phase when HCT116 colorectal cancer cells are treated with 30 μ M of um-PEA for 24 hours (Pagano et al., 2021). Even though the two error bars overlap, implying less reliability, the singular astrix (*) indicates that this increase is to a 95% certainty level, supporting the conclusion that PEA induces cell cycle arrest. Additionally, there was a significantly smaller percentage of cells in the DNA synthesis phase

(S), suggesting an issue occurring with DNA synthesis. When a DNA fragmentation assay was conducted, a distinguishable DNA ladder in gel electrophoresis was visible (Pagano et al., 2021), indicating DNA damage. Therefore the cell cycle arrest induced by um-PEA can potentially cause apoptosis in the colon cancer cells.

Reducing Tumour Cell Migration

Um-PEA was also discovered to reduce tumour cell migration when a scratch assay was conducted (Pagano et al., 2021). A scratch assay is conducted in vitro by creating a “scratch” in a cell monolayer and capturing images at regular intervals during the cells’ migration to close the scratch (Liang et al., 2007). Comparing the images allows one to calculate the migration rate of the cells (Liang et al., 2007). Tumour cell migration is a significant process in metastasis, which is the development of secondary malignant growths. Therefore, um-PEA’s ability to delay this process could be useful in treatments.

Figure 3.1 - photographs of in vitro scratch assay performed on HCT116 and Caco-2 cells (Pagano et al., 2021)

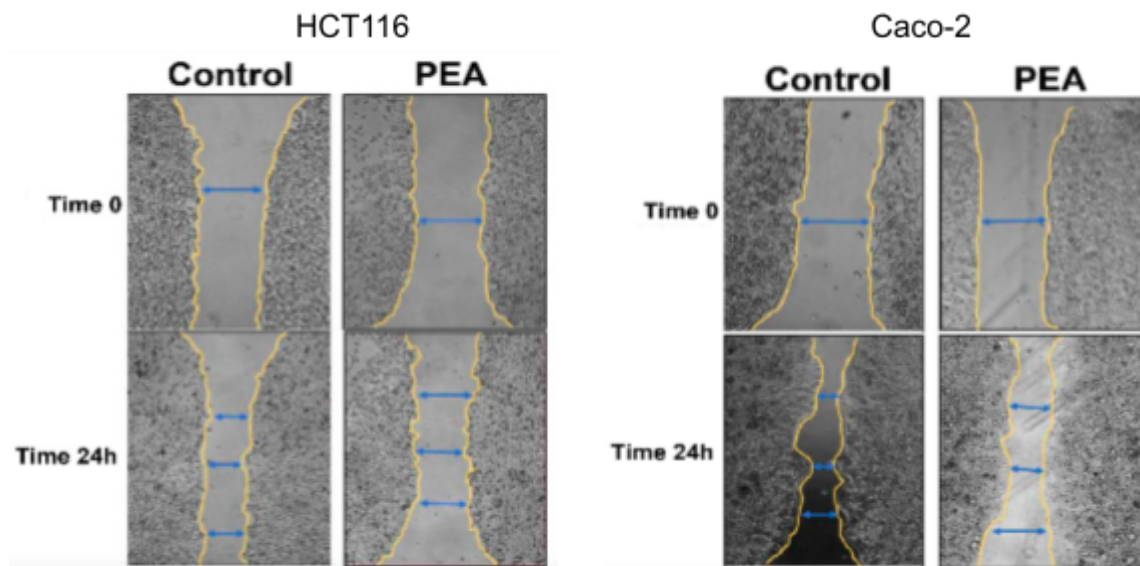
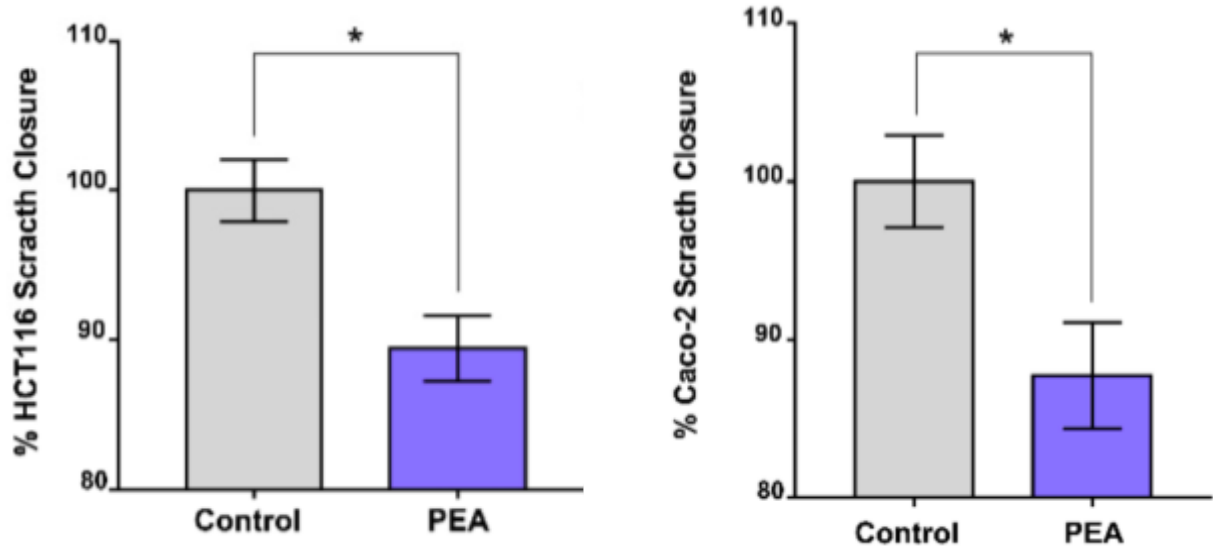


Figure 3.1 shows that for both the HCT116 and the Caco-2 colorectal cancer cell lines, adding 30 μ M of um-PEA results in a wider scratch after 24 hours, suggesting that PEA plays a role in inhibiting the migration of tumour cells. This figure only depicts one trial for each cell line which reduces the reliability of the results as there is no average to mitigate the effects of random errors on migration rate. Yet, the study mentions taking 4 trials for each treatment (Pagano et al., 2021) which can be seen when examining Figure 3.2, the trend is corroborated by the fact that, in both cell lines, there was a greater percentage of scratch closure for the control compared to the cells treated with um-PEA. This percentage decrease is quite significant, going

from 100% closure in the control to 90% and 88% for the HCT116 and Caco-2 cells respectively.

Figure 3.2 - Graph portraying the percentage of closure 24 hours after Scratch Assay for both HCT116 and Caco-2 cell lines (Pagano et al., 2021)



This reduction in tumour cell migration could be because of the depletion of gelatinase A (MMP-2) expression in colorectal cancer cells treated with um-PEA (Pagano et al., 2021). MMP-2 is thought to correlate with tumour spread and a study showed that mice that were MMP-2-deficient had reduced tumour progression (Reunanen and Kähäri, 2013). This supports the notion that PEA can reduce tumour cell migration as a decrease in MMP-2 expression slows down tumour progression.

Potential Applications of Palmitoylethanolamide in the Medical Field

This section will explore how PEA can be used in the medical field to assist with cancer treatments, focusing particularly on its potential role in chemotherapy and radiotherapy.

Palmitoylethanolamide in Chemotherapy

In addition to PEA's intrinsic anti-tumour activity, PEA can also aid in managing side effects induced by chemotherapeutic agents (Hesselink, 2013). An example of this is with the drugs bortezomib and thalidomide. Both these drugs are used to treat multiple myeloma (National Cancer Institute, 2023) and nearly always induce a reversible length-dependent sensory-motor, predominantly axonal, large-fiber polyneuropathy (Hesselink, 2013), a type of neuropathy that affects the feeling and movement of multiple nerves by causing structural damage to the axons (National Cancer Institute, 2023), which are the long projections of the nerve cells that transmit signals. Since it is length-dependent, this affects the longer nerves (legs and feet) first and to a greater degree than shorter ones. This polyneuropathy can be dose-limiting (Hesselink, 2013) as oncologists have to reduce the concentration of dosage and extend treatment time to reduce this side effect. The cumulative neuropathy incidence rate for the combination of both drugs has led to significant delays with a reported ~60% of treatments going overtime (Hesselink, 2013).

In a clinical trial, patients suffering from multiple myeloma added PEA to their cytostatic regime for two months (Hesselink, 2013). This cytostatic regime included 1.3 mg of bortezomib injections twice a week and a daily 50-200 mg of thalidomide tablets (Hesselink, 2013). All patients were exhibiting clear signs of neuropathy and neuropathic pain at the beginning of the trial but after 2 months pain was significantly reduced and blinded examiners found that all neurophysiological measures were significantly improved (Hesselink, 2013), depicting PEA's potential role in assisting chemotherapy. The neurophysiological measures used were Laser

Evoked Potentials (LEPs), Sensory Nerve Action Potentials (SNAPs), and Compound Muscle Action Potentials (CMAPs) (Hesselink, 2013).

Table 1 - Additional information on the processes of each neurophysiological measure and its applications for testing the presence of reversible length-dependent sensory-motor, predominantly axonal, large-fiber polyneuropathy

Neurophysiological Measure	How it works	How it tests for polyneuropathy
LEPs	Measures the cortical evoked potential to selective activation of nociceptive peripheral nerve fibres by applying a brief laser pulse to the skin which stimulates the small, unmyelinated and thinly myelinated fibres responsible for pain sensation, providing insight into the function of the nociceptive pathways (Vollono et al., 2009).	LEPs can be used to evaluate one's sensitivity to pain, which is reduced in nerves affected by polyneuropathy.
SNAPs	SNAPs measure the electrical response of a sensory nerve to stimulation. It is the sum of action potentials from individual sensory nerve fibres. They assess the function of the larger, myelinated A β -fibers responsible for light touch and vibration sensation (Crone and Krarup, 2013).	Diminished or absent SNAPs indicate dysfunction of these sensory nerve fibres (Crone and Krarup, 2013), which is characteristic of large-fibre, sensory polyneuropathy.
CMAPs	CMAPs measure the electrical response of a muscle to stimulation of its innervating motor nerve. It is the sum of motor unit action potentials in the muscle and the compound sensory action potential. They provide information about the integrity of the motor nerve fibres and the neuromuscular junction (Crone and Krarup, 2013).	Reduced CMAP amplitudes suggest impairment of motor nerve function, as seen in motor polyneuropathy.

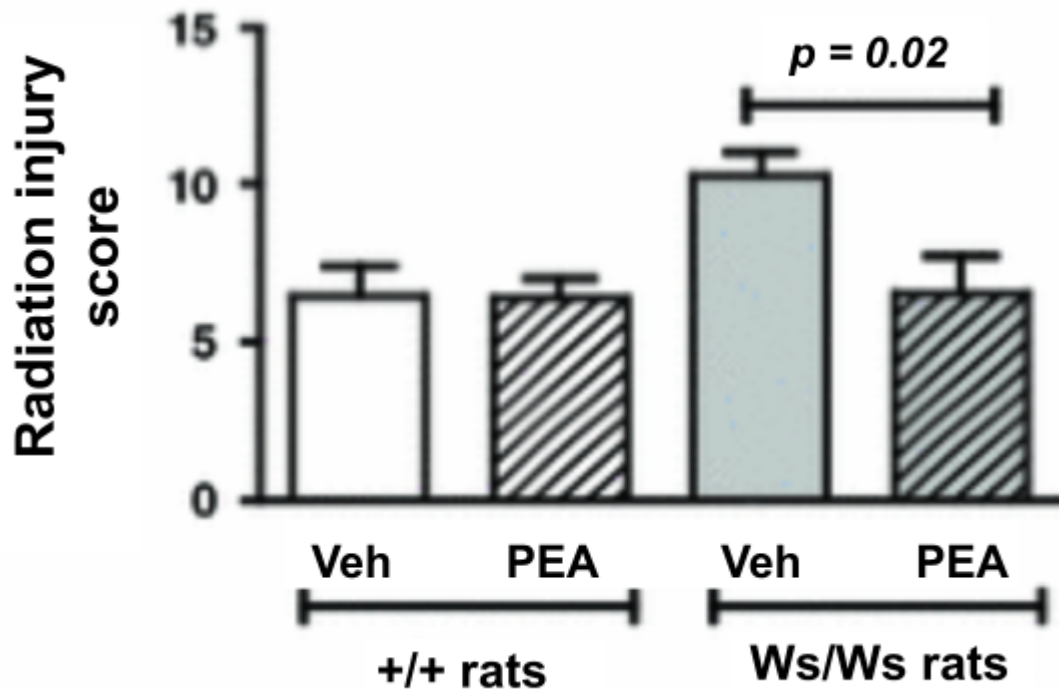
Moreover, in a paper from 1975, PEA was described to aid in the chemotherapeutic treatment of leukemic rats (Hesselink, 2013). In these experiments, the chemotherapy dose could be increased due to the cytoprotective effects of PEA, as the chemical reduced the dose-limiting side effects of various combinations of cisplatin vincristine, cyclophosphamide and methotrexate (Hesselink, 2013). There was also significantly less side effect related mortality in rats treated with PEA, showing the clear add-on effect of PEA on chemotherapy (Hesselink, 2013).

Palmitoylethanolamide in Radiotherapy

Radiotherapy uses high-energy particles or waves (x-rays, gamma rays, electron beams, or protons) to destroy or damage cancer cells (American Cancer Society, 2019). Radiotherapy works by creating small breaks in the DNA inside cells, stopping cancer cells from undergoing mitosis and thus causing them to die (American Cancer Society, 2019). This is similar to the cell cycle arrest that PEA induces, suggesting the PEA can be used to enhance the anti-tumour effects of radiotherapy. PEA's anti-proliferative effects and ability to reduce tumour cell migration can also aid in radiotherapies aim to shrink tumour sizes.

According to a 2014 study, PEA can reduce the severity of intestinal radiation mucositis, a common dose-limiting side effect of radiation therapy for abdominal cancers (Wang et al., 2014). Intestinal radiation mucositis is a common side effect of cancer treatments involving radiation. It is inflammation of the mucosa, the mucous membranes that line the entire gastrointestinal tract or chemotherapy (Cleveland Clinic, 2022). Mucositis is temporary and heals on its own, but it can be painful and carries certain risks (Cleveland Clinic, 2022). Mast cells and neuroimmune interactions are known to regulate the severity of intestinal radiation mucositis, so the study aimed to investigate the effect of PEA in a mast cell-competent (+/+) and a mast cell-deficient (Ws/Ws) rat model of intestinal radiation injury (Wang et al., 2014) by treating rats either with a vehicle or PEA from 1 day before until 2 weeks after radiation (Wang et al., 2014).

Figure 4.1 - the effect of PEA (10 mg/kg/d) on radiation-induced intestinal injury in +/+ rats and Ws/Ws rats (Wang et al., 2014).



As seen in Figure 4.1, Ws/Ws rats sustained more severe intestinal structural injury compared to +/+ rats. However the addition of PEA reduced structural radiation injury in Ws/Ws rats, but not in +/+ rats. Since this reduction for Ws/Ws rats has a $p=0.02$ and there are no overlaps in the error bars, this conclusion can be deemed statistically significant. This suggests that PEA can be used to reduce the effects of intestinal radiation mucositis and act as an adjunct to radiotherapy, but only for mast cell-deficient patients.

Conclusion

In conclusion, PEA has many intrinsic anti-cancer effects (anti-proliferation, inducing cell cycle arrest, and reducing tumour cell migration) however, there has been no indication that PEA can act as a cancer treatment alone. Instead, PEA can assist current treatments in their goals to eradicate cancer cells, for example, PEA's intrinsic anti-cancer effects can be used to enhance the function of chemo- and radiotherapy. However, PEA's most useful potential function in cancer treatment could be its ability to reduce dose-limiting side effects. This would allow oncologists to increase the concentration and duration of cancer treatments, allowing for a better chance of recovery. However, this may be more useful in chemotherapy as PEA can reduce the severity of reversible length-dependent sensory-motor, predominantly axonal, large-fiber polyneuropathy. Since this polyneuropathy stems from common chemotherapeutic agents, PEA's applications in chemotherapy could be more widespread. Whereas with radiotherapy, PEA only reduces intestinal radiation mucositis in mast cell-deficient rats, meaning that when used on human patients, it may only have an effect if they are mast cell-deficient. Intestinal radiation mucositis is also only a common side effect of radiation for abdominal cancers, so PEA might not be as useful in reducing side effects from other targeted radiotherapies.

References

American Cancer Society (2019). *How Radiation Therapy Is Used to Treat Cancer*. Available at: <https://www.cancer.org/cancer/managing-cancer/treatment-types/radiation/basics.html>.

(Accessed: 4 July 2024)

Clayton, P., Hill, M., Bogoda, N., Subah, S., and Venkatesh, R. (2021). *Palmitoylethanolamide: A Natural Compound for Health Management*, International Journal of Molecular Sciences, 22(10). doi: 10.3390/ijms22105305

Cleveland Clinic (2022). *Mucositis*. Available at:

<https://my.clevelandclinic.org/health/diseases/24181-mucositis> (Accessed: 4 July 2024)

Crone, C. and Krarup C. (2013). *Chapter 6 - Neurophysiological approach to disorders of peripheral nerve*, ScienceDirect, 115 pp. 81-114. doi:

<https://doi.org/10.1016/B978-0-444-52902-2.00006-0>

De Petrocellis, L., Bisogno, T., Ligresti, A., Bifulco, M., Melck, D., and Di Marzo, V. (2002). *Effect on cancer cell proliferation of palmitoylethanolamide, a fatty acid amide interacting with both the cannabinoid and vanilloid signalling systems*, Blackwell Science Fundamental & Clinical Pharmacology, 16(4), pp.297-302. doi: 10.1046/j.1472-8206.2002.00094.x

Di Marzo, V., Melck, D., Orlando, P., Bisogno, T., Zagoory, O., Bifulco, M., Vogel, Z., and De Petrocellis, L. (2001). *Palmitoylethanolamide inhibits the expression of fatty acid amide hydrolase and enhances the anti-proliferative effect of anandamide in human breast cancer cells*, Biochemical Journal, 358 (1), pp.249–255. doi: <https://doi.org/10.1042/bj3580249>

Gencor (2023a). *Levagen®*. Available at: <https://www.gencorpacific.com/ingredients/levagen>
(Accessed: 26 June 2024)

Gencor (2023b). *Scientifically Proven to be Effective: Levagen®, Levagen®+* Available at:
https://cdn.prod.website-files.com/5f85d0c81b6f5da2719b6b03/6511aaf0d9ac85ffaf605059_06.01.23_Levagen%20Sell%20Sheet.pdf (Accessed: 26 June 2024)

Hesselink, JMP. (2013). *Palmitoylethanolamide: A Useful Adjunct in Chemotherapy Providing Analgesia and Neuroprotection*, Chemotherapy, 2(3) pp. 121. doi:10.4172/2167-7700.1000121

Liang, CC., Park, A. and Guan, JL. (2007) *In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro*, Nature Protocols, 2, pp.329–333. doi:
<https://doi.org/10.1038/nprot.2007.30>

National Cancer Institute (2023). *A to Z List of Cancer Drugs*, The National Institutes of Health. Available at: <https://www.cancer.gov/about-cancer/treatment/drugs> (Accessed: 3 July 2024)

Pagano, E., Venneri, T., Lucariello, G., Cicia, D., Brancaleone, V., Nani, M.F., Cacciola, N.A., Capasso, R., Izzo, A.A., Borrelli, F., and Romano, B. (2021) *Palmitoylethanolamide Reduces Colon Cancer Cell Proliferation and Migration, Influences Tumor Cell Cycle and Exerts In Vivo Chemopreventive Effects*, Cancers, 13(8):1923. doi: <https://doi.org/10.3390/cancers13081923>

Rankin, L. and Fowler, C.J. (2020). *The Basal Pharmacology of Palmitoylethanolamide*, International Journal of Molecular Sciences, 21(21), pp.7942. doi:
<https://doi.org/10.3390/ijms21217942>.

Reunanen N. and Kähäri VM. (2013). *Matrix Metalloproteinases in Cancer Cell Invasion*. In: Madame Curie Bioscience Database. Austin (TX): Landes Bioscience; Available at: <https://www.ncbi.nlm.nih.gov/books/NBK6598/> (Accessed: 2 July 2024)

Vollono, C., Vigeveno, F. and Valeriani, M. (2009). *Somatosensory system excitability in migraine*, ScienceDirect, 19(1), pp. S22-S27. doi: <https://doi.org/10.1016/j.paed.2009.05.030>

Wang, J., Zheng, J., Kulkarni, A., Wang, W., Garg, S., Prather, PL. and Hauer-Jensen, M. (2014). *Palmitoylethanolamide regulates development of intestinal radiation injury in a mast cell-dependent manner*, Dig Dis Sci, 59(11), pp. 2693-2703. doi:10.1007/s10620-014-3212-5