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Investigation of the Effects of the Endogenous Cannabinoid Anandamide on Luminal A Breast Cancer Cell Line MCF-7

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ABSTRACT

The present study was carried out to investigate anti-tumoral effects of Anandamide (AEA) in luminal A breast cancer cell line MCF-7. Cell viability was measured by MTT assay and cell index was measured by xCelligence DP analyzer system. The Feulgen method was used to determine the mitotic index parameter, and the ^{3}H-Thymidine method was used to determine the labeling index parameter. The apoptotic index parameter was determined using a fluorescent dye DAPI. The results of this study showed that 25 µM Anandamide concentration was the optimum concentration for MCF-7 cells. While this concentration decreased the proportion of cells in the mitotic phase and synthesis phase, it increased the proportion of apoptotic cells.

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Introduction

Breast cancer continues to be an important health problem among women around the world (1). Among all molecular subtypes, luminal type A is the most widespread type of breast cancer (2).

It was suggested that cannabinoids could be used for cancer treatment, with the understanding of the ability of these compounds to kill tumor cells. After the discovery that cannabinoids suppress tumor growth in the early 1970s, studies have shown that cannabinoids induce apoptosis in different types of cancer cells, thereby suppressing the proliferation of tumor cells (3-8).

Components of the endocannobinoid system have been identified as pharmacological targets for cancer therapy. Anandamide was one of the first lipids discovered to be synthesized endogenously at cannabinoid receptors (9, 10).

Cannobinoids were previously used for palliative treatment in cancer patients (11, 12). A new target for cancer therapy has been proposed, with *in vitro* and *in vivo* studies revealing the antitumor properties of these compounds (12).

Cannabinoids target the endocannabinoid system, thereby affecting various cellular processes required for cancer development such as cell cycle arrest, apoptosis, proliferation, invasion, metastasis and angiogenesis (13-15).

In this current study, it was aimed to show the anticancer effects of the endogenous cannabinoid Anandamide on MCF-7 cells originating from luminal A breast cancer.

Materials and methods

Cell Culture

MCF-7 cells were cultured RPMI-1640 (Sigma) with 10% FBS (Gibco Lab.), Streptomycin (Ulugay), Penicillin (Pfizer), Amphotericin B (Sigma) at 37ºC containing 5% CO_2.

Anandamide Concentrations

25 µM, 50µM and 75 µM Anandamide (Tocris, UK) concentrations were arranged by diluting of 1 mM stock solution.

Mitochondrial Enzyme Activity Assay: Cell Viability (MTT)

Cell viability of MCF-7 cells was determined MTT assay as previously reported (16).

Cell Index

Cell viability of MCF-7 cells was determined by

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real time cell analyzer system xCelligence DP described in previous studies (17). Measurement of cell index values was continued for 72 hours after Anandamide application.

Mitotic Index (MI)

Mitotic index of MCF-7 cells was determined according to Feulgen method in previous studies (18). Cells treated with optimum Anandamide concentration were fixed with Carnoy's fixative at the end of the experimental periods. Then Feulgen method was applied and stained with Giemsa.

Labelling Index (LI)

Labelling index of MCF-7 cells was determined ^{3}H thymidine labelling method in previous studies (18). After labelling, autoradiography was applied.

Apoptotic Index (AI)

Apoptotic index of MCF-7 cells was determined DAPI staining method in previous studies (19). Fluorescent microscope was used to identify apoptotic cells.

Statistical Evaluation

Experimental groups data were compared to unidirectional Anova test. Statistical analyses were performed using GraphPad Prism version 6. (GraphPad Software, San Diego, California, USA). In the tests $p< 0.05$ level of significance was accepted.

Results and discussion

Mitochondrial Enzyme Activity Assay: Cell Viability (MTT)

As a result of application of Anandamide on MCF-7 cells, in order to determine whether it has any effect on mitochondrial dehydrogenase enzyme activity, 25 µM, 50 µM and 75 µM Anandamide concentrations were used for 24 hours in cultured cells.

The absorbance values obtained from the experimental series carried out in parallel with the control group without anandamide were shown in Table 1. These values showed that cell viability decreased depend on concentration.

When these absorbance values were examined, the cell viability decreased to 49,33% at 25 µM concentration; to 44,32% at 50 µM concentration and to 35.53% at 75 µM concentration compared to the control group accepted as 100% for MCF-7 cells (Figure 1). Based on these data, 25 µM Anandamide concentration was determined as the optimum concentration for MCF-7 cells.

Table 1. Absorbance values of mitochondrial dehydrogenase activity (450-690 nm) of MCF-7 cells treated with 25 µM, 50 µM, 75 µM concentrations of Anandamide for 24 h ($p<0.05$).

Experimental Groups	Absorbance Values (450-690 nm)
Control	$448.387 \times 10^{-3} \pm 0.014^{SD}$
25 µM	$221.215 \times 10^{-3} \pm 0.011$*
50 µM	$198.736 \times 10^{-3} \pm 0.009$*
75 µM	$157.176 \times 10^{-3} \pm 0.008$*

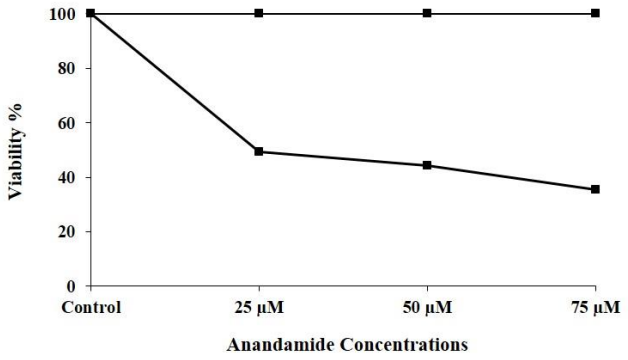

Figure 1. Cell viability values of MCF-7 cells treated with 25 µM, 50 µM, 75 µM concentrations of Anandamide for 24 h ($p<0.05$).

The absorbance values obtained as a result of applying the optimum concentration of 25 µM to MCF-7 cells for 0-72 hours were shown in Table 2. These values showed that the optimum concentration applied significantly decreased cell viability in a time-dependent manner.

Table 2. Absorbance values of mitochondrial dehydrogenase activity (450-690 nm) of MCF-7 cells treated with 25 µM concentration of Anandamide for 0-72 h ($p<0.05$).

Time (Hours)	Absorbance Values (450-690 nm)	
	Control	25 µM
24	$360.680 \times 10^{-3} \pm 0.013^{SD}$	$182.712 \times 10^{-3} \pm 0.002$*
48	$396.118 \times 10^{-3} \pm 0.012$	$112.654 \times 10^{-3} \pm 0.002$*
72	$447.438 \times 10^{-3} \pm 0.015$	$99.123 \times 10^{-3} \pm 0.003$*

Cell Index

When the cell index values obtained as a result of the application of Anandamide at 25, 50 and 75 µM concentrations to MCF-7 cells, compared with the standard curves, it suggested that a cytostatic effect occurs at 25 µM and 50 µM Anandamide

concentrations, and a cytoskeletal effect at 75 µM Anandamide concentrations (Figure 2).

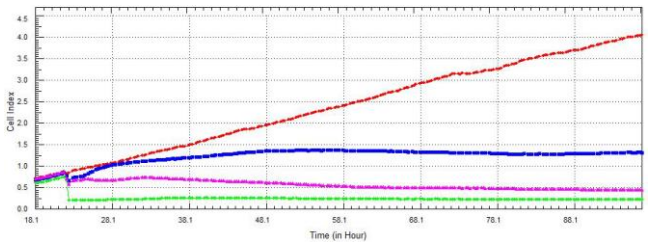

Figure 2. Cell index values of MCF-7 cells treated with 25 µM, 50 µM, 75 µM concentrations of Anandamide obtained from xCelligence Real-Time Cell Analysis (RTCA) system (Red line: Control, Blue line: 25 µM, Pink line 3: 50 µM and Green line 4: 75 µM).

Mitotic Index (MI)

After administration of 25 µM concentration of Anandamide for 0-72 h, the MI rates of MCF-7 cells were shown in Table 3. MI rates of MCF-7 cells decreased significantly over time (Table 3). The differences between the control and all experimental groups were significant ($p<0.05$).

Table 3. Mitotic index values of MCF-7 cells treated with 25 µM concentration of Anandamide for 0-72 h ($p<0.05$).

Time (Hours)	Mitotic Indeks (%)	
	Control	25 µM
24	7.42±0.03^{SD}	3.78±0.02*
48	8.42±0.03	2.96±0.03*
72	8.56±0.04	1.98±0.01*

Labelling Index (LI)

After administration of 25 µM concentration of Anandamide for 0-72 h, the labelling index rates of MCF-7 were shown in Table 4. Labelling index rates of MCF-7 cells decreased significantly time dependent manner (Table 4). The differences between the control and all experimental groups were significant ($p<0.05$).

Table 4. Labelling index values of MCF-7 cells treated with 25 µM concentration of Anandamide for 0-72 h ($p<0.05$).

Time (Hours)	Labelling Indeks (%)	
	Control	25 µM
24	8.18±0.04^{SD}	4.21±0.03*
48	8.21±0.02	3.16±0.05*
72	8.12±0.03	2.96±0.03*

Apoptotic Index (AI)

After administration of 25 µM concentration of Anandamide for 0-72 h, the AI worths of MCF-7 were shown in Table 5. AI worths of MCF-7 cells increased significantly time dependent manner (Table 5). The differences between the control and all experimental groups were significant ($p<0.05$).

Table 5. Apoptoic index values of MCF-7 cells treated with 25 µM concentration of Anandamide for 0-72 h ($p<0.05$).

Time (Hours)	Apoptotic Indeks (%)	
	Control	25 µM
24	8.33±0.02^{SD}	16.26±0.08*
48	9.62±0.01	20.42±0.09*
72	10.22±0.05	27.45±0.13*

The aim of this study was to investigate the antiproliferative effect of the endogenous cannabinoid anandamide on luminal A breast cancer using different cell kinetic parameters. The results presented in this study confirm the antiproliferative effects of anandamide on the luminal A breast cancer cell line MCF-7.

Results from various studies have shown that Anandamide has an antitumoral effect in different cancer models. These effects are manifested by cell cycle arrest, decreased cell viability, induction of cell death types such as apoptosis, necrosis, and autophagy (20).

In a study investigating the antiproliferative effects of anandamide in human hepatocellular carcinoma cells, anandamide was shown to inhibit the proliferation of the hepatocellular cell line Huh7 by arresting the cell cycle in the G_1 phase and promoting apoptosis (21). Anandamide has affected breast cancer to varying degrees. *In vitro* and *in vivo* studies have shown that this compound inhibits angiogenesis. Studies on the triple negative breast cancer cell line MDA-MB-231 have shown that anandamide inhibits endothelial cell proliferation promoted by MDA-MB-231 cells (22). An anandamide analog has been observed to suppress the growth of thyroid cancer *in vivo* by inhibiting angiogenesis (23). Anandamide has been shown to increase apoptosis, suppress motility ability in the glioblastoma cell line U251 and also inhibit tumor growth *in vivo* (24). Anandamide has also been shown to suppress the epithelial mesenchymal transition, which is an important mechanism in the invasion and metastasis process of cancer cells (25).

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Interest conflict

The authors declare that they have no conflict of interest.

Author's contribution

All authors responsible for the manuscript equally.

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