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Investigation of anticancer effects of Meteorin like protein on different human cancer cell lines

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Abstract

Aim: Cancer is one of the biggest health problems threatening humanity, and the fight against cancer continues with different methods worldwide. Despite advances in cancer prevention and treatment, the low success rate and tumor recurrence make the discovery of new alternative agents important. Adipokines are among those known to be associated with cancer. The relationship of the peptide-structured Meteorin like protein (Metrnl), a member of the adipokine family, discovered in 2012, with cancer remains a mystery. This study was conducted to examine the cytotoxic effects of Metrnl on human ovarian, prostate, colon and breast cancer cell lines.

Materials and Methods: In the study, ovarian (A2780), human prostate (LNCaP), colon (Caco-2) and breast cancer (MCF-7) cell lines were used. After the cells were incubated with 1, 5, 10, 50, 100 and 200 ng/mL Metrnl for 24 h, the cytotoxicity level was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) assay method. determined. Comparisons between groups were made with the Kruskal Wallis H-Test.

Results: Concentrations of Metrnl significantly reduced cell viability in A2780, LNCaP, Caco-2 and MCF-7 cell lines ($p<0.05$).

Conclusion: These results show that Metrnl may have anticarcinogenic activity on A2780, LNCaP, Caco-2 and MCF-7 cells, but further studies are needed on this subject.

Introduction

Cancer is a large group of diseases that can begin in almost any tissue of the body when cells grow and multiply uncontrollably [1]. It is known that one in every 5 people worldwide will develop cancer during their lifetime [2]. According to the report of the International Agency for Research on Cancer, it is reported that there will be an estimated 20 million new cancer cases worldwide and 9.7 million deaths due to cancer in 2022. According to data in 2022, it is reported that the most common type of cancer is lung cancer (approximately 2.5 million), followed by breast (11.6%), colorectum (9.6%) and prostate (7.3%) cancers. It is reported that the rate of ovarian cancer is approximately 1.6% [3]. Recent studies report that these types of cancer are linked to overweight or obesity, and approximately 30% of cancer-related deaths are due to obesity [4]. Obesity, which negatively affects almost all physiological functions of the human body, can cause many serious chronic diseases, including cancer [5]. Obesity, which occurs as a result of the imbalance between energy intake and expenditure, is defined as an excess amount of fat tissue in the body [6]. Adipose tissue is the largest known endocrine organ, and it is thought that mediators released from adipose tissue may be related to cancer, and at the same time, the adipose tissue microenvironment may play roles in carcinogenesis, metastasis development and disease progression [7, 8]. Obesity, characterized by increased fat mass, may cause white adipose tissue (WAT) inflammation [9]. However, obesity, which mediates most of the systemic complications, is a chronic state of subclinical inflammation. Chronic inflammation can cause cancer, inflammatory cells and mediators found in tumor microenvironments are responsible for tumor development and progression. Since chronic inflammation is also associated with obesity, it can cause an increase in cancer prevalence and mortality [10, 11]. When the current literature data is examined, the molecular and physiological mechanisms underlying cancer continue to be investigated with different and current approaches. Since

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it has been associated with increasing obesity rates in recent years, research focuses on adipose tissue and mediators secreted from adipose tissue. For this reason, the effects of adipokines such as leptin and adiponectin on various cancer mechanisms have been examined [12-14]. Considering the high mortality rates in cancer, the search for effective treatment continues, as well as the currently known treatment methods. In this context, newly discovered adipokines, anti-inflammatory agents, hormones and cytokines, which are released from fat tissue and play a role in energy metabolism, have been the focus of attention in recent years.

Meteorin like protein (Metrnl), also known as Meteorin-β, Subfatin, Cometin, and IL-39, is a newly secreted adipokine that exerts pleiotropic effects on inflammation, immunology, and metabolism [15]. Metrnl has been detected in various tissues, including skeletal muscle, brown adipose tissue, testis, macrophages, kidney, liver, heart, stromal cells, spleen and brain [16, 17]. This peptide stimulates gene expression where anti-inflammatory cytokines are produced, causing browning in WAT, which is of great importance in metabolic diseases, and reduces obesity-related insulin resistance by improving fat function [16, 18]. Studies suggest that Metrnl may be related to obesity [19]. On the other hand, in the current literature, the protumor effect of Metrnl, also known as IL-39, on pancreatic cancer cells has been reported to be associated with the decrease of the anti-proliferative molecule p21, and its anti-apoptotic effect has been revealed to be associated with the decrease of pro-apoptotic molecules [20]. In a study, it was reported that Metrnl may be associated with colorectal cancer [21]. There is limited research in the literature focusing on the effects of Metrnl on cancer. The fact that Metrnl is released from fat tissue in the body and that fat tissue has important roles in the development of cancer suggests that Metrnl, which has inflammatory activity, may be related to cancer. It is unknown whether Metrnl plays anti-proliferative or cytotoxic roles on cancer cells. The aim of this study is to determine the cytotoxic effects of Metrnl on human ovarian (A2780), prostate (LNCaP), colon (Caco-2) and breast cancer (MCF-7) cell lines.

Materials and Methods

Chemicals

Metrnl (Biolegend, San Diego, CA), Fetal Bovine Serum (FBS; Serox, Germany), penicillin-streptomycin (Gibco, USA), dimethyl sulfoxide (DMSO; Merck, Germany), Minimum Essential Medium (MEM) non-essential amino acids (Biological Industries, Israel), insulin (Novorapid, Denmark), 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Serva, Germany), RPMI-1640/DMEM F-12/DMEM-High Glucose medium and other chemicals (Biowest, USA) were used. Distilled water was used throughout all experimental stages. Metrnl to be tested was dissolved in 0.9% isotonic sodium chloride to prepare concentrations of 1, 5, 10, 50, 100, and 200 ng/mL.

Cell culture

This study was conducted in the Department of Physiology laboratories at İnönü University School of Medicine. A2780, LNCaP, Caco-2, and MCF-7 cancer cell lines were used in the study. A2780 and LNCaP cells were cultured in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum (FBS), 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 5 mL of MEM non-essential amino acids solution. Caco-2 cells were cultured in DMEM F-12 medium, and MCF-7 cells were cultured in DMEM High Glucose medium. Both cell lines were supplemented with 10% FBS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 5 mL of MEM non-essential amino acids solution, and 1 mL of insulin. All cells were incubated in 25 cm^2 culture flasks in an incubator (ESCO, Egaa, Denmark), and the medium was changed twice a week. When cells reached confluence, they were detached from flasks using trypsin-ethylenediaminetetraacetic acid (EDTA) solution. To assess cell viability, cells were stained with 0.4% trypan blue and counted under an inverted microscope. Cells with viability above 90% were used for MTT assay analyses [22, 23]. For MTT assay analyses, cells were detached from flasks using trypsin-EDTA solution when they reached confluence, transferred to 96-well plates ($15x10^3$ cells per well) and incubated for 24 h. After incubation, Metrnl at concentrations of 1, 5, 10, 50, 100, and 200 ng/mL was added to the 96-well plates and left for a further 24-hour incubation period (All incubation stages were performed in an incubator set at 37°C with 5% CO_2).

MTT assay

Cytotoxicity was assessed using the widely used enzymatic test method known as the MTT assay. The method is based on the principle of MTT dye cleaving the tetrazolium ring. MTT is actively absorbed by viable cells and catalyzed by mitochondrial succinate dehydrogenase to formazan, a blue-purple, water-insoluble product. Formazan formation is observed only in live cells containing active mitochondria. This is considered an indicator of cell viability and is correlated with the number of living cells [24, 25]. First, a working solution of 0.5 mg/mL MTT was prepared from a stock MTT solution in sterile phosphate buffer solution. After incubation with Metrnl for 24 hours, 50 µL of MTT solution was added to each well containing A2780, LNCaP, Caco-2, and MCF-7 cells. The plates were then incubated for 3 hours in the incubator. After incubation, the MTT solution was removed from the plates, and 100 µL of DMSO was added to each well to dissolve the cells' formazan crystals. The optical densities of the cells were measured at 550 nm using an ELISA reader (Thermo MultiskanGo, USA) [26].

The average absorbance values obtained from the readings of control wells were considered as 100% viable cells. The values from wells treated with solvent and Metrnl were compared with the control wells to calculate the percentage of viability [27-29]. These experiments were conducted at least ten times on separate days [30].

Statistical analysis

Statistical analysis was performed using IBM SPSS software program for Windows (SPSS Inc., Chicago, IL). Between-group comparisons of research results were conducted using the Kruskal-Wallis H-test. When statistically

significant differences were found between the groups, multiple comparisons were conducted using the Mann Whitney U test with Bonferroni correction (all values of $p < 0.05$ were considered statistically significant).

Results

In vitro cytotoxic activity

It was determined that Metrnl had a reducing effect on the viability rates of all cell lines when applied to A2780, LNCaP, Caco-2 and MCF-7 cancer cell lines at concentrations of 1, 5, 10, 50, 100 and 200 ng/mL.

The percentage changes in cell viability with Metrnl application are presented in Figure 1. Application of 200 ng/mL Metrnl in the A2780 cell line reduced cell viability and this decrease was found to be statistically significant ($p<0.05$).

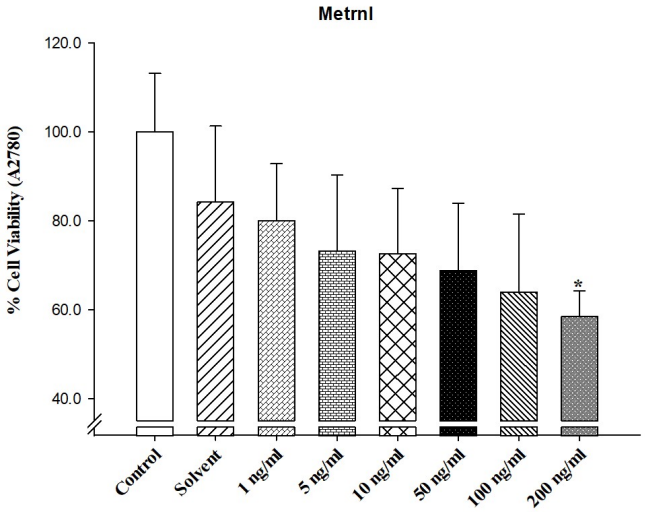

Figure 1. Cell viability of A2780 cells after Metrnl application (*According to the solver, it represents statistical significance, *p<0.05, Metrnl: Meteorin like protein).

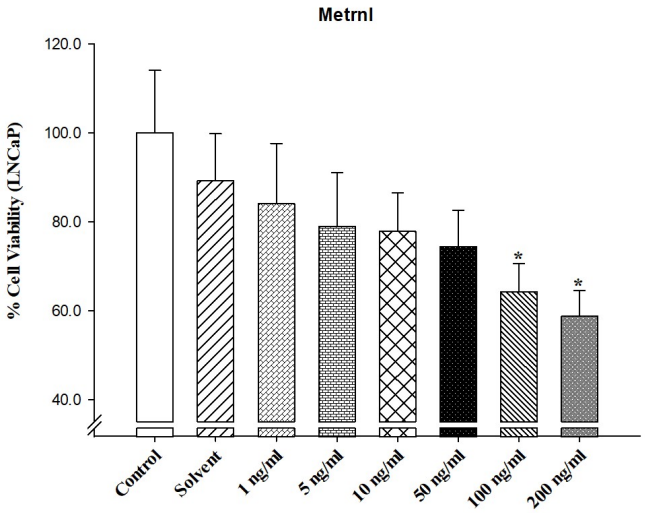

Figure 2. Cell viability of LNCaP cells after Metrnl application (*According to the solver, it represents statistical significance, *p<0.05, Metrnl: Meteorin like protein).

The % changes in the viability of LNCaP cells 24 hours after the application of 1, 5, 10, 50, 100 and 200 ng/mL concentrations of Metrnl are shown (Figure 2). Metrnl reduced cell viability at all applied concentrations compared to the control and solvent groups. On the other hand, this decrease was found to be statistically significant, especially at concentrations of 100 and 200 ng/mL ($p<0.05$).

After incubating Caco-2 cells with Metrnl (1, 5, 10, 50, 100 and 200 ng/mL) for 24 hours, its effect on cell viability is shown in Figure 3. All concentrations of Metrnl decreased cell viability, and especially the decrease at 200 ng/mL was

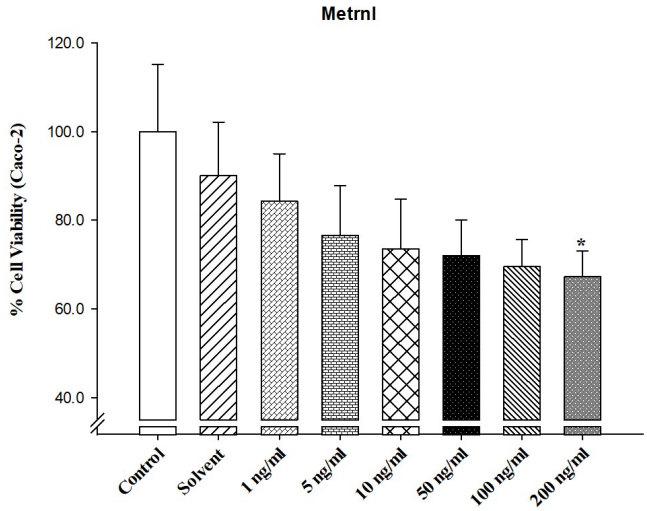

Figure 3. Cell viability of Caco-2 cells after Metrnl application (*According to the solver, it represents statistical significance, *p<0.05, Metrnl: Meteorin like protein).

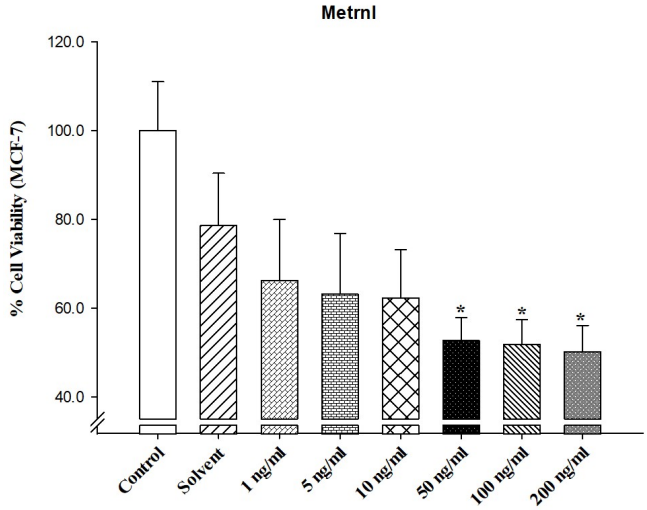

Figure 4. Cell viability of MCF-7 cells after Metrnl application (*According to the solver, it represents statistical significance, *p<0.05, Metrnl: Meteorin like protein).

found to be statistically significant ($p<0.05$).

The change in MCF-7 cells after incubating concentrations of Metrnl (1, 5, 10, 50, 100 and 200 ng/mL) for 24 hours is shown in Figure 4. All concentrations of Metrnl reduced cell viability compared to the control and solvent group. This decrease, especially in the groups where 50, 100 and 200 ng/mL Metrnl was applied, was found to be statistically significant ($p<0.05$).

Discussion

Cancer, which is characterized by the uncontrolled proliferation of abnormal cells, is defined as "a wound that never heals" [31]. Cancer, which is not a communicable disease but causes three out of every 10 global premature deaths (30.3% of those in the 30-69 age group), is among the three leading causes of death in this age group in 177 of 183 countries [32]. It is reported that there are approximately 240 thousand cancer cases in our country and approximately 12.5% of them result in death [3]. In recent years, the relationship between cancer and obesity, defined as increased fat mass in the body, has attracted attention [4]. The relationship between hormones and cytokines secreted from adipose tissue and cancer has been revealed, albeit partially [13].

Studies report that adipokines are associated with various types of cancer [12-14, 33]. It is known that leptin, one of these adipokines, plays a role in the development and progression of breast cancer [12, 33]. In another study, it was proven that leptin was associated with worse prognosis in patients treated with platinum compounds combined with paclitaxel/docataxel [34]. It has been found that the level of adiponectin is low in individuals with colorectal cancer [35]. However, there is evidence that adiponectin has a suppressive effect on breast cancer cells [36]. Resistin, another adipokine, is reported to promote the formation of ovarian cancer [37]. Tekin S and his colleagues found that apelin-13 increased the viability of different prostate cancer cell lines [27]. Considering the findings in the current literature, we think that there may be a strong connection between adipokines and cancer. Anti-inflammatory adipokines are reported as promising therapeutics for cancer research. In our study, we report the potential cytotoxic effects of the anti-inflammatory adipokine Metrnl on different cancer cell lines. Metrnl showed cytotoxic effects when applied at different concentrations.

The number of studies examining the relationship between Metrnl and cancer is quite low. In a study examining the possible role of Metrnl in doxorubicin-induced cardiotoxicity, it was reported that it did not change the tumoricidal effect of doxorubicin in 4T1 breast cancer cell line and tumor mice [38]. In another study, it was reported that the immunoreactivity of Metrnl increased at the cellular level in invasive ductal breast cancer [39]. It has been determined that the level of Metrnl is high in the colon carcinoma tissue of individuals with colon cancer [40]. All these findings bring to mind the idea that Metrnl may be effective in cancer.

In our study, 1, 5, 10, 50, 100 and 200 ng/mL concentrations of Metrnl were applied to A2780, LNCaP, Caco-2 and MCF-7 cells. In these experiments, we report that Metrnl reduced cell viability in all cell lines. These findings show that Metrnl, an anti-inflammatory adipokine, has cytotoxic activity on cancer cells and is a potential candidate for use in cancer therapy. Additional and more comprehensive studies are needed to understand the mechanism of action of Metrnl on cancer cells.

Ethical approval

Ethical approval was not required as it was a cell culture study.

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