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Cytotoxic Activity of Carrageenan on Malignant MCF-7 Breast Cancer and The Non-Malignant SVCT Breast Epithelial Cell Lines

Karragenan'ın Malign MCF-7 Meme Kanseri ve Malign Olmayan SVCT Meme Epitel Hücre Dizileri Üzerindeki Sitotoksik Aktivitesi

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ÖZET

AMAÇ: Son yapılan çalışmalar deniz alglerinden elde edilen sülfatlanmış polisakkaritlerin birçok biyolojik ve fizyolojik aktivitesi olduğunu göstermektedir. Kırmızı deniz alglerinden elde edilen sülfatlanmış bir polisakkarit olan kappa-karragenanın da çeşitli kanser hücre hatlarında anti-proliferatif etki gösterdiği bilinmektedir. Bu çalışmada kappa-karragenanın, iki farklı hücre hattı, malignant özellikte MCF-7 meme kanseri hücre hattı ve non-malign SVCT meme epiteli hücre hatları üzerindeki sitotoksik etkisi in vitro modelde incelenmiştir.

GEREÇ VE YÖNTEM: Karragenanın 1000 µg/ml başlangıç dozu olacak şekilde ve her hücre için kendi besiyerinde üç farklı konsantrasyonu hazırlanmıştır (Dilution I: 1000 µg/ml; Dilution II: 250 µg/ml; Dilution III: 62,5 µg/ml). Hücreler deney gruplarında belirlenen dozlarda karregenan ile inkübe edilmiştir. Karragenan içermeyen besiyerinde inkübe edilen hücreler de kontrol grubu olarak alınmıştır.

BULGULAR: Hücre canlılığının ölçülmesi için MTT [3-(4,5-dimetiltiyazol-2-il]-2,5-difenil-tetrazolyum bromür] analizi yapılmıştır. Hücre morfolojisi, acridine orange (AO)/propidium iodide (PI) florasan boyama yöntemi ile incelenmiştir. Çalışmamızda elde ettiğimiz sonuçlar, karragenanın hem malign hem de non-malign hücre hatları üzerinde sitotoksik etkiye neden olduğunu göstermektedir.

SONUÇ: Sonuç olarak karragenın malign ve non-malign hücreler üzerinde sitotoksik bir etkisinin olduğunu söylemek mümkündür. Ancak bu etki yüksek karregenan dozları kullanıldığında belirgin olarak görülmekte olup; MCF-7 kanser hücre hattında bu etki daha düşüktür. MCF-7 hücre hattının kültür sırasında ortaya çıkabilecek olası spontan fenotipik ve genotipik değişiklikler de göz önüne alındığında, karreganın anti-tümör etkisinin olabileceği ancak bununla birlikte daha MCF-7 dışında farklı hücre hatları kullanılarak yapılacak çalışmalar ile bu sonuçların desteklenmesi gerektiği düşünülmektedir.

Anahtar Kelimeler: Karragenan, sitotoksite, MCF-7, SVCT, MTT

ABSTRACT

OBJECTIVE: Recent studies have shown that sulfated polysaccharides obtained from marine algae have many biological and physiological activities. Kappa-carrageenan, a sulfated polysaccharide obtained from red marine algae, is known to have anti-proliferative effects in various cancer cell lines. In this study, the cytotoxic effect of kappa-carrageenan on two different cell lines, namely the malignant MCF-7 breast cancer cell line and the non-malignant SVCT breast epithelial cell lines, was investigated in an in vitro model.

MATERIALS AND METHODS: Three different concentrations of carrageenan were prepared for each cell in its own medium, with an initial dose of 1000 µg/ml (Dilution I: 1000 µg/ml; Dilution II: 250 µg/ml; Dilution III: 62.5 µg/ml). Cells were incubated with carrageenan at the doses set for each experimental group. Cells were incubated in a carrageenan-free medium comprised the control group.

RESULTS: To measure cell viability, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) analysis was performed. Cell morphology was investigated by the acridine orange (AO)/propidium iodide (PI) fluorescent staining method. The present results indicated that carrageenan caused cytotoxic effects on both malignant and non-malignant cell lines.

CONCLUSION: Considering that the different phenotypic features of the subtypes of the MCF-7 cell line may affect cell viability and cell proliferation, cell selection should be performed very carefully in cytotoxicity studies. We suggest that using the MCF-7 cell line for cytotoxicity experiments needs to contemplate this important phenomenon for further experimental setups.

Keywords: Carrageenan, cytotoxicity, MCF-7, SVCT, MTT

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INTRODUCTION

For the last 30 years, various polysaccharides or protein-polysaccharide components have been isolated from fungi, yeast, some plants, and algae. These polysaccharides draw attention especially due to their immunomodulatory properties and anti-tumor activities (1). Sulfated polysaccharides from marine algae have specific properties that play a role in ionic regulation in cells (1). Recent studies have shown that these sulfated polysaccharides obtained from marine algae have many biological and physiological activities (1).

Carrageenan is the general name given to a group of sulfated polysaccharides obtained from red marine algae (2). Carrageenan, obtained from seaweeds belonging to the Rhodophyceae family, is a 15%-40% sulfated polygalactan molecule with a molecular weight of about 100 kDa (2). There are several types of carrageenan such as λ, κ, ι, ε, and μ, all of which contain 22%-35% sulfate groups. The position and number of ester sulfate groups in the molecule determine the properties and function of the molecule (2). Studies have shown that carrageenan has anti-thrombotic, anti-inflammatory, anti-viral, and anti-tumor activities (3). The molecular structure of carrageenan determines its function (4). Kappa-carrageenan has been shown to have an anti-proliferative effect in various cancer cell lines and an anti-tumor activity in in vivo studies (5, 6, 7).

In this study, the cytotoxic effect of kappa-carrageenan, a type of carrageenan obtained from red algae, on two different cell lines, namely, the malignant MCF-7 breast cancer cell line and non-malignant SVCT cell line were investigated in an in vitro model.

MATERIAL & METHODS

Preparation of cell cultures

MCF-7 and SVCT cell lines were prepared in 96-well culture dishes with an initial cell count of 20,000 cells/200 µl in six replicates. The MCF-7 cell line was incubated in 95% air-5% CO_2 at 37C° for 24 hours under standard culture conditions in Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F12 (Biochrom AG, Germany) containing 10% fetal bovine serum (FBS) (Biochrom AG, Germany); and, the SVCT cell line was incubated in 95% air-5% CO_2 at 37C° for 24 hours under standard culture conditions in DMEM/Ham's F12 (Biochrom AG, Germany) containing 10% fetal bovine serum (FBS) (Biochrom AG, Germany), 5 µg/ml hydrocortisone, and 10 µg/ml insulin.

Preparation of the test material and application to cell lines

Carrageenan (Merck, Germany) stock solution was prepared at room temperature as 20 mg/20 ml in serum-free medium and sterilized by passing through a 0.22 µm filter. Based on the studies in the literature (8,9), three different concentrations were prepared for each cell in its own medium with an initial dose of 1000 µg/ml (Dilution I: 1000 µg/ml; Dilution II: 250 µg/ml; Dilution III: 62.5 µg/ml). In the control group, cells were incubated in their own medium not containing carrageenan.

Examination of cell viability

To measure cell viability, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) analysis was performed. MTT method is a standardized procedure assaying cell viability and widely used in cytotoxicity tests. In our previous studies, we used this method to measure cell viability in different in vitro cytotoxicity models (10, 11, 12). This colorimetric method assesses the ability of viable cells to form MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) formazan by the mitochondrial enzyme succinate dehydrogenase. At 24 hours of the incubation of the cells in 96-well plates, the medium was taken out and 12.5 µL of MTT (Sigma-Aldrich, Germany) solution in 100 µL of FBS-free DMEM/F12 was added to each well. Cell culture plates were wrapped with aluminum foil and incubated for 4 hours. At the end of the incubation, the MTT solution was removed and the reaction was stopped by adding 100 µL of isopropyl alcohol (Amresco Inc., USA). Cell viability was measured with an ultraviolet (UV)-visible spectrophotometer (EZ Read 400 Microplate Reader, Biochrom) at a wavelength of 560 nm as the absorbance value.

Assessment of cell morphology

Acridine orange (AO)/propidium iodide (PI) fluorescent staining was used for the assessment of cell morphology. At the 72nd hour of incubation, the medium on the cells was removed and the cells were incubated for 20 seconds by adding AO/PI (Sigma-Aldrich, Germany) (600 mg/ml AO, 600 mg/ml PI, ratio of 1:1) without fixation. The cells were then washed twice with phosphate buffer saline (PBS) (Sigma-

Aldrich, Germany) for 10 seconds. Next, the cells were examined under fluorescence microscopy. Dead cells were evaluated by counting the red cells with fragmented nuclei. The AO/PI-stained cells were observed under a narrow band fluorescein (FITC) filter (520–560 nm) in green color, and the PI-stained cells were observed under a rhodamine filter (510–560 nm) as stained red.

Statistical Analysis

Statistical analyses were performed using the IBM SPSS statistics 23 program. The difference caused by carrageenan on cell viability was analyzed with the Kruskal Wallis test, which is a non-parametric test. The Bonferroni test, which is a post-hoc test, was used for the difference between the groups. Any p-value <0.05 was considered statistically significant.

RESULTS

Assessment of Cell Viability

Cell viability was evaluated for all three dilutions at 72 hours of incubation and compared with the control group. The results are given in Table 1 together with the p values. As seen in Table 1, cell viability was lower in both cell lines compared to the control group at the highest concentration (Dilution I). The difference was not significant in the MCF-7 cell line (p>0.05) but significant in the SVCT cell line (p<0.05). When the MCF-7 and SVCT cell lines were compared with each other for all concentrations, no significant difference in cell viability was observed in all three dilutions (Table 2).

Table 1. *Cell viability in MCF-7 and SVCT cell lines*

Dilutions	MCF-7			SVCT		
	Mean	SD	P	Mean	SD	P
I	0.338	0.025	> 0.05	0.318	0.019	< 0.05
II	0.374	0.036	> 0.05	0.350	0.048	> 0.05
III	0.372	0.031	> 0.05	0.413	0.054	> 0.05
Control	0.358	0.054	-	0.483	0.069	-

SD: Standard deviation p values are given by comparing with the control group

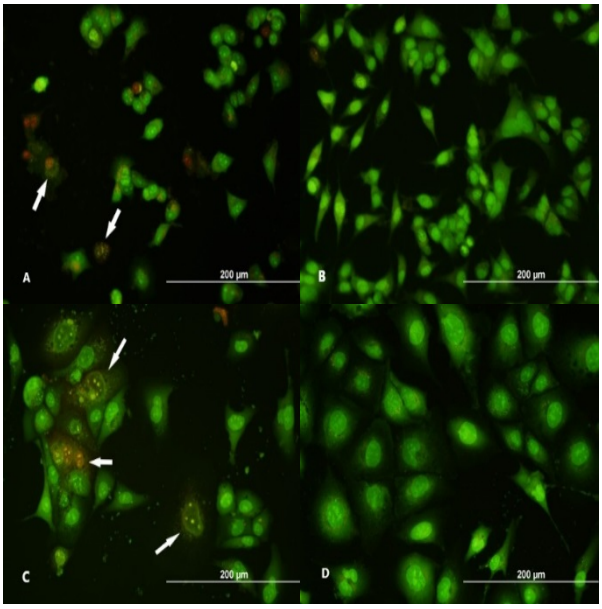
Table 2. *Comparison of cell viability in MCF-7 and SVCT cell lines*

	Dilutions		
	I	II	III
P values (MCF-7 / SVCT)	> 0.05	> 0.05	> 0.05

Assessment of cell morphology

When the cell morphologies were examined, although it was observed dead cell in both cell line, the cell morphology in the SVCT cell line was different than the control group. Furthermore, it was observed that the cells moved away from the epithelial morphology and assumed a more rounded appearance, and at the same time, nuclear condensation became evident. In some cells, DNA fragmentation occurred and the nucleus was stained in yellow-red-orange colors (Figure 1).

Figure 1. *AO/PI staining of cell lines exposed to test material (Dilution I: 1000 µg/ml) and control group at 72 h of incubation.*



(A) *MCF-7 cell line (Dilution I) (20x);* ***(B)*** *MCF-7 cell line (Control) (20x);* ***(C)*** *SVCT cell line (Dilution I) (20x) and* ***(A)*** *SVCT cell line (Control) (20x). (White arrows indicate rounded and degenerated cells).*

DISCUSSION

Cell lines are extremely important in in vitro models for studies on the diagnosis and treatment of breast cancers at the molecular level (13). There are various studies in the literature examining the anti-tumor effects of carrageenan on different cell lines. Luo et al. showed that λ-carrageenan suppressed cell proliferation by altering the tumor microenvironment in B16-F10 melanoma and 4T1 breast cancer cell lines (14). In another study, it was shown that λ-carrageenan suppressed cell proliferation in MDA-MB-231 cells and caused apoptotic cell death (8). The effects of different types of carrageenan on different cell lines are also investigated (15). In a study, lambda carrageenan was shown to have an anti-proliferative effect on SH-SY5Y and MCF-7 breast cancer cell lines, like kappa carrageenan (9). In another study, it was shown that both carrageenan types suppressed cell division in HeLa cell line, and their anti-

tumor effect was emphasized (16). In another study, the effects of low molecular weight lambda and kappa carregenans on cytotoxic and cytokine expression on esophageal cancer cell lines KYSE30 and FLO1 cells were investigated (17).

MCF-7 cell line is a breast cancer cell line with estrogen (+), prolactin (+) and human epidermal growth factor receptor-2 (HER2(+)) features; therefore, it is frequently used in studies on the effects of anti-cancer drugs (18). The MCF-7 cell line was first obtained in 1973 by Dr. By Soule et al. at the Michigan Cancer Foundation (MCF) from a metastatic tumor that developed 7 years later in a woman with breast cancer, and it was named MCF-7 for this reason (13). The SVCT cell line is a human immortalized breast epithelial cell line obtained by creating the immortalized genotype of healthy breast tissue cells with the Simian virus 40 (SV40) (19). The SVCT cell line has the characteristic of non-malignant breast epithelium (20). Therefore, these two cell lines were selected in our study in order to demonstrate the cytotoxic effect of carrageenan on malignant (MCF-7) and non-malignant (SVCT) cells.

In our results, although the cell viability at the highest carrageenan concentration in MCF-7 cells was lower than the control group, this difference was not statistically significant ($p>0.05$). However, in the SVCT cell line, which is obtained from healthy breast tissues, the cytotoxic effect was more pronounced than in the MCF-7 cell line, and cell viability was lower than in the control group, which was a statistically significant difference ($p<0.05$). When the MCF-7 and SVCT cell lines were compared with each other for all concentrations, the difference in cell viability was not statistically significant ($p>0.05$). The MCF-7 cell line is a weakly aggressive non-invasive cell line and shows weak metastatic properties (21, 22). However, the results we obtained in our study showed that, despite this feature, cell viability in the MCF-7 cell line was similar to that of SVCT, a non-malignant mammary epithelial cell line.

When the cell morphologies were examined, although it was seen in both cells, the cell morphology in the SVCT cell line differed compared to the control group; cells moved away from epithelial morphology and became more rounded; nuclear condensation became evident; some cells had DNA fragmentation; and, the nuclei were stained in yellow-red-orange colors. This shows that there is a morphology of the onset of apoptosis in cells. Apoptosis, which is programmed cell death, is seen as a promising approach in anti-cancer drug treatments due to its mechanism of formation (23, 24). In our study, cells with this morphology were observed in both cell types at the highest dose of carrageenan (Dilution I)]. However, in order to make a definitive assessment on this subject, it is inevitable that more detailed studies are required to show the pathway of apoptosis and the signaling mechanisms by which carrageenan acts in this process.

Although MCF-7 cells are mostly of the same phenotype in an in vitro culture, in some cases subtypes of cells with different phenotypes emerge within the same population, and there is a variation in the gene expression of surface receptors and signaling pathways in these cells (13). Differences in cell proliferation also occur in these subtypes of cells, and the likelihood of these phenotypic changes increases with the changes in physicochemical conditions (13). In long-term cultures, these cells show a profile similar to those of breast cancer types that are clinically resistant to anti-estrogen and anti-aromatase treatments. However, the main problem here is that this phenotypic change in subtypes also leads to changes in surface receptors and signaling mechanisms (25). These changes may lead to results that may affect cell viability, proliferation, and morphology in different ways.

In conclusion, the results obtained in the present study showed that carrageenan caused cytotoxic effects on both malignant and non-malignant cell lines. Therefore, carrageenan can be considered a new approach in anti-cancer treatments. On the other hand, not a very effective cytotoxic effect was observed in MCF-7 cancer cells. Considering that the different phenotypic characteristics of the subtypes of this cell line can affect cell viability and cell proliferation, it was concluded that it is extremely important to know the genotypic and phenotypic characteristics of the cells used in in vitro cytotoxicity studies and to determine whether it will undergo a change in the culture in the end.

Etik: Bu çalışmanın etik kurulu alınmıştır.

Ethics committee approval had been taken.

Yazar katkı durumu: Çalışmanın konsepti; MS, HSA, EK, ÖAG, dizaynı; MS, HSA, EK, ÖAG, literatür taraması; MS, ÖAG, verilerin toplanması ve işlenmesi; MS, HSA, ÖA, EK, ÖAG, istatistik; MS, ÖA, ÖAG, yazım aşaması; MS, HSA, EK, ÖAG

Author contribution status: The concept of the study; MS, HSA, EK, ÖAG, design; MS, HSA, EK, ÖAG, literature review; MS, ÖAG, collecting and processing data; MS, HSA, ÖA, EK, ÖAG, statistics; MS, ÖA, ÖAG, writing phase; MS, HSA, EK, ÖAG

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