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Investigation of the Effects of Omeprazole, a Proton Pump Inhibitor, on Apoptotic Gene Regions on Human Fibroblast Cells

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Abstract

Proton pump is the first-line treatment for progression of stomach acids such as peptic ulcer disease, gastroesophageal reflux disease, and nonsteroidal anti-inflammatory drug-induced mucosal damage (Strand, Kim, and Peura 2017). Omeprazole, a proton pump inhibitor, is commonly used to treat gastroesophageal reflux disease, peptic ulcers, and other stomach conditions. OME inhibits H+/Na+ ATPase in the stomach view, ensuring proton secretion of the gastric lumen and reducing gastric acid secretion. Omeprazole's effect temperatures and support have not yet been defined in detail. In addition, prolonged duration of this situation brings with it some side effects (such as gastrointestinal diseases, bone fractures, vitamin and mineral deficiencies) (Fontecha-Barriuso et al. 2020). Therefore, it is of great importance to elucidate the complement in detail for the effectiveness of this transaction process. In our expansion study in this direction, the effectiveness of this pressure on cell viability on fibroblast conditions where Omeprazole was applied in various regions was determined by viability test, while the effectiveness on the expression disruption of apoptotic genes with this pressure was carried out by quantitative real-time PCR analyses. It is aimed to use the additive to better preserve the obtained results of Omeprazole with the data obtained from the analysis results.

Keywords: Omeprazole, Proton pump inhibitors, Apoptosis, Cell Viability

Introduction

Proton pumps are generally known as carrier proteins of the cell membrane and play a role in regulating the p assage of ions within the cell, especially protons, thro-ugh the cell membrane. These proteins are particularly a therapeutic target for drugs known as proton pump inhibitors (PPIs), which are used to control stomach acid secretion. These drugs reduce the output of ions and molecules within the cell by inhibiting the proton pumps in the cell membrane. PPIs keep pH values

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inside the cell low by pumping proteins out of the cell membrane. PPIs reduce stomach acid production by inhibiting the protein pumps in the cell membrane that produce stomach acid. With this mechanism, they are used in the treatment of gastroesophageal reflux disease, stomach ulcers and other stomach disorders. PPIs are also used in the treatment of stomach ulcers caused by the bacterium Helicobacter pylori (Der 2003; Atkins and Sekar 2013).

Omeprazole (OME), one of the proton pump inhibitors frequently used in the regulation of stomach acid secretions and peptic ulcer treatments in today's conditions, is a pharmacological agent preferred by a high patient population. OME drug provides a decrease in the amount of gastric acid secretion through reversible inhibition of the H + / K + ATPase enzyme (Fontecha-Barriuso et al. 2020), and with this mechanism, it is used for the effective treatment of diseases such as gastroesophageal reflux disease, Zollinger-Ellison syndrome, and peptic ulcers of the gastrointestinal tract (Walt et al. 1983). However, information about the potential effects of this drug at the cellular level and on apoptotic gene regions is limited, and long-term use of proton pump inhibitors brings with it some side effects (such as gastrointestinal infections, bone fractures, vitamin and mineral deficiencies). Therefore, it is of great importance to elucidate the molecular mechanisms of these drugs in detail in order to increase their effectiveness in the treatment process.

This study aimed to detail the effects of OME on different apoptotic gene regions on fibroblast cells, which are located in the connective tissue and are involved in critical processes such as growth, repair and renewal of this tissue, regulation of the immune response and control of inflammation, and to elucidate the mechanisms of these effects at the cellular level. The results obtained aim to understand the long-term genetic effects of OME at the cellular level and to reveal the possible mechanisms of potential side effects resulting from the use of this drug.

Materials and Methods

Cell culture

Supply of Human Fibroblast Cell Line: The human dermal fibroblast (HDF) used in this study is available in our Erzurum Technical University Molecular Cancer Biology laboratory and was obtained from a liquid nitrogen tank at -196° C.

Preparation of Cell Culture Medium and Passaging of Cells (Subculturing): For human fibroblast cell lines, 100 ml of ready-to-use cell culture medium was prepared by adding 10 ml of fetal bovine serum (FBS), 1 ml of penicillin-streptomycin (Pen-Strep) solution, and 89 ml of ready-made Dulbecco's Modified Eagle Medium (DMEM) high-glucose cell culture medium. The prepared media were stored at +4 °C in 50 ml vials. The cells were first kept in DMEM solution containing 10% FBS, 1X L-Glutamine, and 1X Penicillin/Streptomycin, and then they were planted in T25 flasks with 15 ml of medium in an oven with 5% carbon dioxide at 37 °C. Cells were dissociated with trypsin every 2-3 days to produce cells for four weeks. All cell culture studies were carried out in a sterile laminar air-flow cabinet.

Cell Line Passaging: Frozen human fibroblast cells were allowed to thaw in a 37°C water bath for 1-2 minutes. Then, the cell suspension tube content was transferred to a 15 ml centrifuge tube, 3-4 ml of warm fresh medium was added, and centrifugation was performed at 1500 rpm for 5 minutes. The supernatant was removed, and the pellet was resuspended in fresh medium and transferred into a 25 cm^2 flask containing 4 ml medium.

Cell Incubation Conditions: Human fibroblast cell lines were incubated in a cell culture medium at 37 °C with 5% carbon dioxide (CO_2) and 95% humidity in an incubation oven (Esco) under sterile conditions in 25 cm2 flasks, allowing the cells to proliferate.

Drug Treatment: Proton pump inhibitor (OME) was prepared at different determined concentrations. Various concentrations of this inhibitor (5 mg/mL, 10 mg/mL, 20 mg/mL, 40 mg/mL, 60 mg/mL and 80 mg/mL) were added to the plates containing fibroblast cells. For the control group, a plate containing untreated cells was prepared.

Cell Cytotoxicity Analysis

MTT Analysis: For cell viability analysis, 5,000 cells were planted in each well of 96 plates, and the cell-seeded plate was kept in a carbon dioxide incubator for 24 hours. Then, OME was applied to the cells, and a 6-hour incubation was provided. The medium on the surface of those cells was removed with a pipette, and OME applications were made for 24 hours. After 24 hours of protein inhibitor application, 10 µl of the prepared thiazolyl blue tetrazolium bromide solution was added to 90 µl medium and above, and following the addition of this solution, the 96-well plate was placed in the incubator and kept for 3 hours. Then, this cell medium was removed, and 100 µl of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) solvent solution was added and kept in the incubator for 20 minutes. The absorbance values of the incubated cells were measured at 570 nm with a microplate reader spectrophotometer in 3 repetitions. The applied dose and the % cell viability curve were determined with the help of the Microsoft Excel program, and the 50% inhibitory concentration (IC50) value was calculated with a logarithmic slope graph.

Quantitative Real-Time (Qrt) PCR Analysis: Total RNA isolation from the cell culture medium was performed using a commercial kit (Ambion RNA Mini Kit, USA). The spectrophotometric method determined the concentration and purity of the isolated total RNA. For this purpose, the Nanodrop device designed for DNA/RNA measurements and capable of measuring in micro volumes was used (EPOCH Take3 Plate, Biotek), and RNA concentration and purity were automatically calculated with the software on the device according to 260/280 nm values and the results were determined as ng/µl. The concentration of RNA isolated from approximately 500 thousand cells was measured as 100 ng/µl and was found to be pure.

cDNA Synthesis: cDNA synthesis from total RNA was performed using the High-Capacity cDNA Reverse Transcription Kit enzyme. Then, each reaction was carried out with 100 ng/µl of RNA, and cDNA synthesis was achieved with a Veriti 96 Well Thermal Cycler (Applied Biosystems) according to temperature values in accordance with the literature. The amount of cDNA was determined by nanodrop spectrophotometry (EPOCH Take3 Plate, Biotek) and stored at -20˚C.

Uploading Stage to Real-Time PCR Device: The mRNA expression of AKT-3, PIK3CA, BCL-2, BAX, MTOR, and GAPDH genes in cells obtained from the HDF cell line after drug applications was quantified using the Taq Man Gene Expression Master Mix kit. The Qiagen Rotor-Gene Real-Time PCR System (Rotor-Gene Q 5plex HRM System) device was used for amplification and quantification. For expression analysis, the mixture was prepared in the proportions given in Table 1.

Table 1. Preparation of the necessary mixture for expression analysis

Content	Volume
2X SYBR Master Mix	5 µl
Forward Primer	0.5 µl
Reverse Primer	0.5 µl
cDNA sample	3 µl
PCR-grade water	9.5 µl

Real-time PCR was carried out under the conditions of 3 minutes enzyme activation at 95 ºC, 5 s denaturation at 95 ºC (40 cycles), and 10 s amplification at 60 ºC (40 cycles). Primers used for the RT-PCR procedure are given in Table 2.

Table 2. Gene sequences used for RT-PCR analysis

Genes	Forward Primer	Reverse Primer
BAX	*CGCATCCTGAGGCACCGG*	*TTTCATCCAGGATCGAGCAGGG*
BCL-2	*CGCATCCTGAGGCACG*	*TTTCATCCAGGATCGAGCAGGG*
AKT-3	*GGAAGAATGGACAGAAGCTATTCCA*	*TCCACTTGCCTTTCTCTCGAAC*
MTOR	*GTCAGTGGGACAGCATGGAAG*	*CCCATATGCCCGACTGTAACTC*
PIK3CA	*TGGATGCTTCACAGGGCTTTCT*	*TATCTTGCCGTAAATCATCCCCCA*

Results

Cytotoxicity Analysis: In the evaluation of MTT analysis results, the highest cell viability value was found in the control group, in which no application was made. In contrast, the lowest cell viability value was determined in the group in which 5 mg/mL OME was applied. In the evaluation of other groups, it was seen that applications between 10 and 80 mg/mL had cell viability values at approximately certain rates, while the cell viability value of the DMSO group with a concentration of 30% applied to the cells decreased significantly. Analysis results are presented in Figure 1**.**

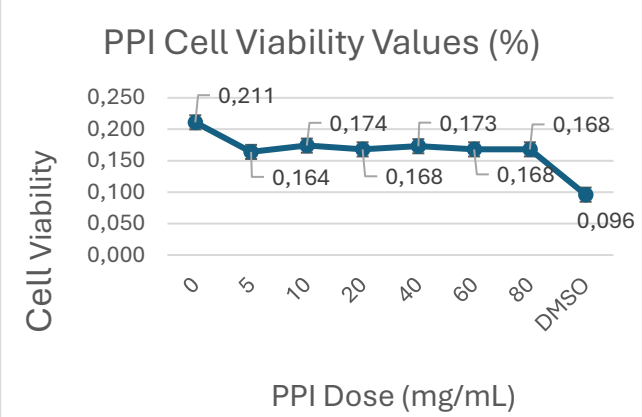

Figure 1. Cytotoxicity analysis values of HDF human fibroblast cell lines incubated for 24 hours after OME application at different doses.

Real Time-PCR Analyses

BCL-2 mRNA Expression: BCL-2 mRNA expression analysis determined that the expression level of the Control group in HDF cells was higher than the other groups at the end of the 24-hour incubation period. In addition to the control group, the 5 mg/mL group is another group with high expression levels(Figure2).

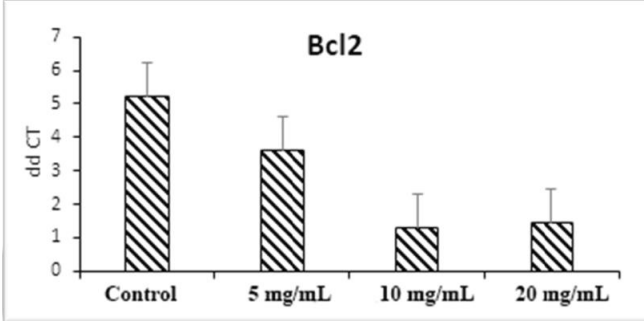

Figure 2. BCL-2 gene expression values of different doses of proton pump inhibitors in the HDF cell line incubated for 24 hours.

PIK3CA mRNA Expression: In PIK3CA mRNA expression analysis, 20 mg/mL expression levels in HDF cells were found to be higher than other groups after 24 hours of incubation (Figure 3).

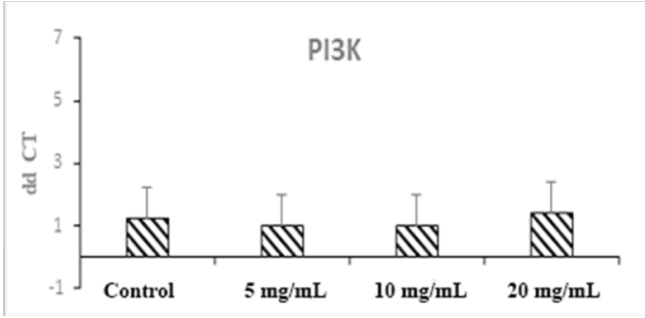

Figure 3. PIK3CA gene expression values of HDF cell lines incubated for 24 hours with the application of different doses of proton pump inhibitors.

BAX gene mRNA Expression: In BAX mRNA expression analysis, expression values were determined to be high in the 20 mg/mL group in HDF cells incubated for 24 hours (Figure 4).

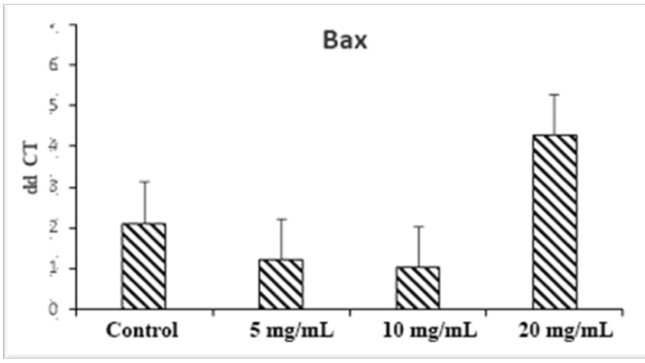

Figure 4. BAX gene expression values of HDF cell lines incubated for 24 hours with the application of different doses of protein pump inhibitors

AKT-3 Gene mRNA Expression Values: In AKT-3 mRNA expression analysis, the highest expression level in HDF cells incubated for 24 hours was determined as the control group. Other groups were determined with expression values close to each other (Figure 5).

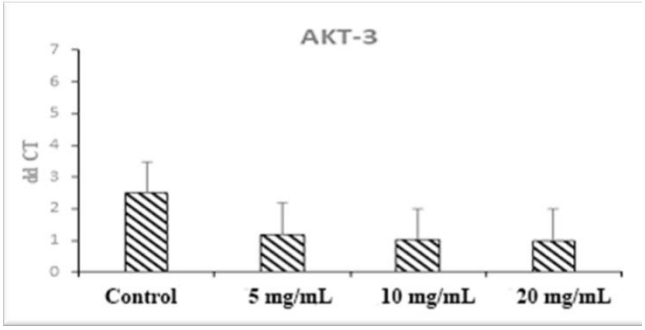

Figure 5. AKT-3 gene expression values of HDF cell lines incubated for 24 hours with different doses of protein pump inhibitors.

mTOR Gene mRNA Expression Values: In mTOR mRNA expression analysis, the highest expression level in HDF human fibroblast cells incubated for 24 hours was determined as the 20 mg/mL group. Close to this group, the group showing the highest expression value is 10 mg/mL. Other groups were determined with expression values close to each other (Figure 6).

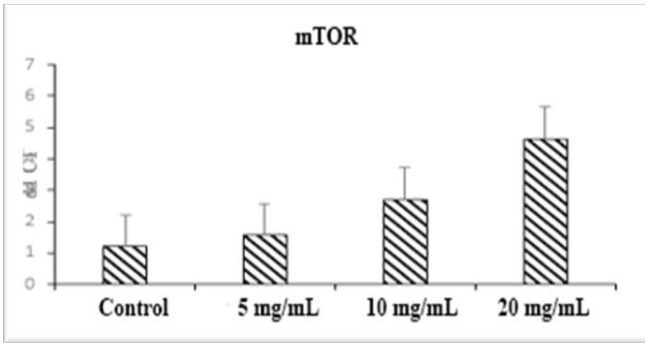

Figure 6. mTOR gene expression values of HDF cell lines incubated for 24 hours with the application of different doses of protein pump inhibitors

Discussion

Omeprazole is a pharmacological agent belonging to the PPI group and is widely used to suppress gastric acid secretion. This medication is known for its effectiveness in treating acid-related disorders such as reflux, stomach ulcers, and gastroesophageal reflux disease. However, long-term use or high doses of omeprazole may cause various adverse effects (Source). This study aimed to determine the cytotoxic and apoptotic effects of PPI use on human healthy cells (HDF).

There are several studies on the effects of OME on cell viability, but the scientific data on this subject is not fully clear and some results are contradictory. The effects of OME on cell viability have generally been examined in in vitro studies, and these studies have revealed different results (Calleja et al. 2021). It has been stated that OME, especially at high concentrations, can inhibit the growth and proliferation of cells and trigger the programmed cell death process of cells called apoptosis (Sun et al. 2019). However, there is no clear evidence as to whether these effects occur in clinical use and what their effects are on the human body. In our study, it was determined that OME causes cell death depending on the dose increase (Omeprazole Induces Apoptosis in Normal Human Polymorphonuclear Leucocytes - E. Capodicasa, P. Cornacchione, B. Natalini, A. Bartoli, S. Coaccioli, P.

Marconi, L. Scaringi, 2008 n.d.). Additionally, changes in the expression of BCL-2, BAX, mTOR, PI3K and AKT-3 genes as a result of application of different doses of OME on HDF human fibroblast cells were analyzed. As a result of this analysis, it was shown that BCL-2, which has anti-apoptotic protein properties, was expressed lower in HDF human fibroblast cells compared to the control group after application of different doses of OME. It has been shown that the expression of the BAX gene, which induces apoptosis, increases as a result of the application of OME at high concentrations. The detected expression levels of these two genes can be interpreted as the application of high concentrations of OME on HDF human fibroblast cells increases the apoptosis mechanism in these cells. When the expression of PI3K and AKT-3 genes was examined in different concentrations of OME applications on this cell, although they were expressed in lower amounts compared to the control group, a relatively significant increase could not be detected according to the application doses. In addition, mTOR gene expression levels showed a significant increase compared to the control group as a result of high dose application of OME. This increase can be interpreted as promoting cell growth in HDF human fibroblast cells. However, the effects of OME on cell viability remain a subject of research and results may vary. Therefore, further studies are needed to draw a clear conclusion regarding the effects of OME on cell viability. In particular, the data obtained from clinical studies on this subject are of great importance.

Research on the effects of OME on apoptosis has revealed various results. Apoptosis plays an important role in many processes such as normal cell development, tissue homeostasis and correction of pathological conditions. Some in vitro studies have suggested that high concentrations of OME may cause apoptosis in cells or affect the apoptosis pathway (Murali and Reif 2023). In these studies, it was found that OME could increase cell death or inhibit the growth and proliferation of cells, especially in cancer cell lines (Hou et al. 2018). OME acts on these mechanisms by reducing acid secretion and inhibiting proton pumps in cells. The conflicting results on this subject reflect the complexity of the effects of OME on cells. Furthermore, it may be difficult to determine the effects of OME on apoptosis in clinical practice because these effects may be influenced by complex factors.

In conclusion, the effects of OME on apoptosis are still an issue under investigation and more studies are required to reach a clear conclusion. Therefore, more data are needed to make a definitive judgment about the effects of OME on apoptosis.

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