The Effect of Melittin on Autophagy Induced by Everolimus in MCF-7 Human Breast Cancer Cells

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Abstract

This study aimed to evaluation of the effect of melittin on autophagy induced by Everolimus in breast cancer cells. MCF-7 cell line was treated with some concentrations of melittin and Everolimus, and according to the IC50, the cells were treated to IC50 dose, higher and lower doses than IC50 in combination or separately. Then, combination index (CI), dose reduction index parameters, and occurrence of autophagy were evaluated. Also, the expression levels of genes related to the autophagy pathway were investigated. The results of this study indicated that melittin and everolimus decreased viability in a concentration- and time dependent manner, and the combined treatment had a synergistic effect. After 24 hr treatment with IC50 concentration, autophagy was decreased significantly in the combined group compared to the group treated with Everolimus (P<0.05). The results of molecular analysis confirmed the data. Melittin reduces the resistance of human breast cancer cells or increases their sensitivity to Everolimus through blocking of autophagy process, and consequently, more breast cancer cells are eliminated.

Introduction

Breast cancer is one of the main causes of death in women. There are various treatments for cancer, such as radiation therapy and chemotherapy. The anti-cancer drugs have two major problems, which are drug resistance and side effects. One of the ways to reduce the problems of using anti-cancer drugs is to use combination treatment, and in this field, materials of natural origin are one of the solutions considered by researchers.

The European Medicines Agency (EMA) has approved Everolimus as an effective drug for treating advanced breast cancers with positive and negative hormone receptors.

The European Medicines Agency (EMA) has approved Everolimus as an effective drug for treating advanced breast cancers with positive and negative hormone receptors. Autophagy can be induced by melittin in hepatocellular carcinoma cells. Considering that both melittin and everolimus substances have anti-breast cancer activities, and the combined effect of these two substances has not been investigated; therefore, we decided to study the simultaneous effect of these two drugs on the autophagy in human breast cancer cell lines.

Methods and Materials

Human breast cancer cells were purchased from the Pasteur Institute of Iran. They were seeded in RPMI medium, 10% Fetal Bovine Serum, and without antibiotics. The cell line was kept in the incubator at a temperature of 37°C, 90% humidity, and 5% carbon dioxide. Cells were treated with melittin (0.01, 0.03, 0.06, 0.12, 0.25, 0.5, 1, 2 µM) and Everlimus (0.01, 0.03, 0.06, 0.12, 0.25, 0.5, 1, 2 µM) for 24 and 48 and 72, and 96 hr for the MTT assay. For other tests, concentrations of IC50 and treatment duration of 24 hr were used.

MTT test was done to measure cell survival. Autophagy assay was done by Acridine orange, which accumulates in acidic organelles in the cell in a pH-dependent manner. The expression levels of Atg-7, Beclin-1, and lC-3 were analyzed by real-time PCR assay. Statistical analysis was done by Tukey's test one-way analysis of variance (P < 0.05 significant) .

Primer Sequences for RT-PCR Analysis

Genes	Primer
Atg-7	
Forward	5-ATTGCTGCATCAAGAAACCC-3
Reverse	5-GATGGAGAGCTCCTCAGCA-3
Beclin-1	
Forward	5-GCCGAAGACTGAAGGTCA-3
Reverse	5-GTCTGGGCATAACGCATC-3
LC-3	
Forward	5-GATGTCCGACTTATTCGAGAGC-3
Reverse	5-TTGAGCTGTAAGCGCCTTCTA-3
GAPDH	
Forward	5-TCCCTGAGCTGAACGGGAAG-3
Reverse	5-GGAGGAGTGGGTGTCGCTGT-3

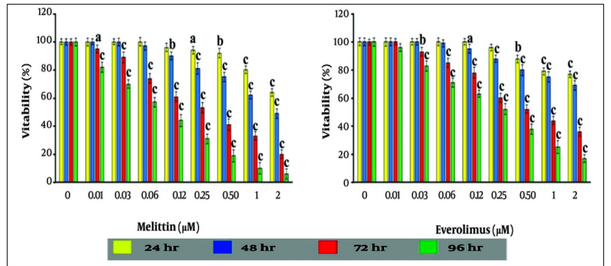
Results

The data of the present study showed that after all four periods of treatment with melittin, cell viability decreased gradually with increasing concentration. After 24, melittin decreased the cell viability significantly in the concentrations of 0.25, 0.5, 1, and 2 µg/mL compared to the control group. After 48 hr, the decrease in survival was significant in concentrations of 0.12, 0.25, 0.5, 1, and 2 µg/mL compared to the control group. After 72 and 96 hr of treatment, the effect of melittin on reducing survival was significant in all groups. IC50 values for melittin were 4.25, 1.62, 0.32, and 0.1 µg/mL for 24, 48, 72, and 96 hr, respectively.

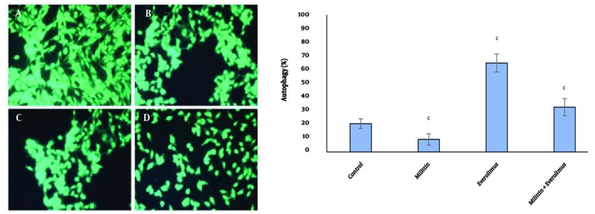
Melittin decreased autophagy-related genes expression . Their expression was decreased significantly in the combination group compared with Everolimus-treated group.

Conclusions

Melittin reduces the resistance of human breast cancer cells or increases their sensitivity to everolimus through blocking of autophagy process, and consequently, more breast cancer cells are eliminated.



The effects of A, milittin; and B, everolimus on Breast cancer cells viability.
The data are expressed as the percentage of control cells as the means ± SEM.
a, P < 0.05; b, P < 0.01; and c, P < 0.01 compared with control.



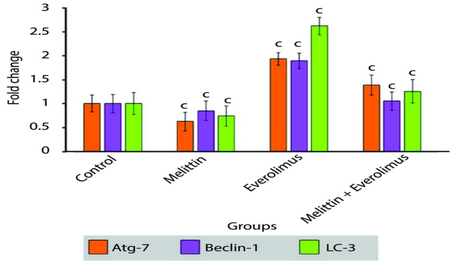
The effects of melittin and everolimus on autophagy in breast cancer cells. A, control cells; B, in the presence of IC_{50} concentration of melittin, C, in the presence of IC_{50} concentration of everolimus; D, in the presence of IC_{50} concentration melittin and of IC_{50} concentration everolimus; and E, columns mean percentage of autophagic cells from three independent experiments. Red dots indicate autophagic vesicles. The data are expressed as the percentage of the control cells as the means± SEM. c, P < 0.01 compared with control

Fraction Affected, Combination Index and Dose Reduction Index Values for Melittin and Everolimus Combination

Co-treatments Groups	Fa	CI	DRI Melittin	DRI Everolimus
1	0.34 ± 0.05	0.96	2	2.11
2	0.55 ± 0.04	0.81	2.47	2.43
3	0.68 ± 0.09	0.92	2.22	2.09
4	0.82 ± 0.07	0.85	2.5	2.2
5	0.89 ± 0.05	0.95	2.29	1.92

Abbreviations: Fa, fraction affected; CI, combination index; DRI, dose reduction index.

[a] Group 1: 1.13 µg/mL of melittin + 1.34 µg/mL everolimus; group 2: 2.26 µg/mL of melittin + 2.69 µg/mL everolimus; group 3: 4.52 µg/mL of melittin +5.38 µg/ml everolimus; group 4: 9.04 µg/mL of melittin + 10.76 µg/mL everolimus; and group 5: 18.08 µg/mL of melittin + 21.52 µg/mL everolimus.



The effects of Milittin and/or everolimus on Atg-7, Beclin-1, and LC-3 expression.
The data are expressed as the percentage of control cells as the means ± SEM. c, P < 0.001 compared with control.

Contact

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