Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

A new target for the treatment of endometrium cancer by succinic acid

Elif Sinem Iplik[1], Tolga Catmakas[2], Bedia Cakmakoglu[2*]

[1] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Istanbul Yeni Yuzyil University, Istanbul, Turkey
[2] Department of Molecular Medicine, Aziz Sancar Institute for Experimental Medicine, Istanbul University, Istanbul, Turkey

Correspondence to: bedia@istanbul.edu.tr

Received September 25, 2017; **Accepted** January 25, 2018; **Published** January 31, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.1.11

Abstract: Endometrium cancer is the most common invasive gynecologic malignancy in developed countries. Succinic acid (CO_2HCH_2-CH_2CO_2H) is a type of dibasic acid that has uncolored crystal. Succinic acid is used in bakery products and aromatized products. It is naturally found in some vegetables. Succinic acid has no adverse effects because it is metabolized by body cells and has a role in the tricarboxylic acid cycle (TCA) as a cycle media component. The TCA cycle and its enzyme components have some crucial roles for basal cell metabolism. Any mistakes, concentration differences in product, or enzyme deficiencies are important within the cell this cycle. In this proposal project, we aimed to investigate the effect of succinic acid at different doses and at different times in an endometrial cancer cell line. The study was performed using methods that determine for apoptosis (for cytotoxicity, WST-1, for caspase enzyme activity, Caspase 3/BCA; apoptotic determination using flow cytometry; Annexin V; to understand mitochondrial membrane potential; JC-1). The results showed that 5 and 10 µM concentration of succinic acid resulted in apoptosis in endometrium cancer; no such effect was seen in the control cell line, which comprised healthy lung cells. According to our results, it is thought that succinic acid would be effective for the treatment of endometrial cancer cell lines, thus providing new data for other areas of cancer research.

Key words: Endometrial cancer; Succinic acid; Anticancer.

Introduction

Cancer is one of the main causes of death worldwide, especially in industrially developed countries. Endometrial cancer is the most frequent of the gynecologic cancers; it is fourth among all cancers.

Endometrial cancer generally occurs in women aged around 61 years, although histologic grade, myometrial invasion, stage, cervical emission, presence of metastasis are considered as prognostic factors. Depending on these factors, treatment options become considerably adjuvant in addition to surgical options (1, 2).

The endometrial cancer process begins with the deformation of balance between cell proliferation and absence of cell apoptosis, and tumor suppression gene or oncogene activation impairment/loss.

Whatever the reason for endometrium cancer, regulations of the diagnosis and treatment options are important for women's health.

Researchers have focused on finding new and specific options for the effective treatment of endometrial cancer. In accordance with this aim, despite chemotherapy and radiotherapy, daily and herbal products have been targeted and are being investigated.

In recent years, herbal and animal-origin products have been investigated and results from vegetables showed some noteworthy effects against the progression of cancer. Consequently, succinic acid has been studied as an antiproliferative agent but its effect has not yet been investigated in many cancer types, including endometrial cancer.

Succinic acid (CO_2HCH_2-CH_2CO_2H) is a type of dibasic acid that has uncolored crystal, and is used in bakery products and aromatized products. It is naturally found in some vegetables (3-5). Succinic acid has no adverse effects because it is metabolized by body cells and has a role in the tricarboxylic acid cycle (TCA) as a cycle media component. The TCA cycle and its enzyme components have some crucial roles for basal cell metabolism (6).

Any mistakes, concentration differences in product, or enzyme deficiencies are important within the cell this cycle.

In our research, we aimed to investigate the effect of succinic acid in different doses and at different times on an endometrial cancer cell line for the first time, using methods that determine for apoptosis (for cytotoxicity, WST-1, for caspase enzyme activity, Caspase 3/BCA; apoptotic determination using flow cytometry; Annexin V; to understand mitochondrial membrane potential; JC-1).

Materials and Methods

Cell culturing components

The endometrium cancer cell line CRL-2923 and healthy lung fibroblastic cell line MRC-5 were obtained from ATCC (American Type Culture Collection, Manassas, VA). The CRL-2923 cell line was cultured in RPMI-1640 medium containing 1% penicillin/streptomycin and 10% fetal bovine serum and also DMEM medium was used for culturing MRC-5 cell lines in the condition of at 37°C in 5% CO_2.

Cytotoxicity

After succinic acid treatment on CRL-2923 and

MRC-5 cell lines, WST-1 cell proliferation assay was performed to find its role on cell viability. Increasing concentrations of succinic acid was obtained on $1x10^4$ cells/well, then incubated at 37ºC in 5% CO_2 for 24, 48, and 72 hrs. When the incubation was completed, 10 µl of WST-1 was treated on cells to measure color change levels. Multiscan ELISA reader (Thermo Fisher Scientific, Germany) was used at 450 nm wavelengths for color development.

Mitochondrial membrane potential

Mitochondrial membrane potential (MMP) is one of the significant sign for apoptosis. That is the reason we have examined the level of losing MMP in response to succinic acid treatment for 48h in CRL29-23 and MRC5 cells. JC-1 Mitochondrial Membrane Potential Detection Kit (Cayman Chemicals, USA) was used to determine the loss of the MMP.

First of all, the cells (5×10^5 cells/2 mL) were treated by succinic acid for 48 hrs. Than, kit procedure was done. Finally, 20 µl of JC-1 dye was added onto the cells and after incubation, the values were calculated to determine the changes in MMP using flow cytometry.

Caspase-3 enzyme activity

We have examined the changes in cellular caspase-3 enzyme activity which is one other important apoptosis sign by using a caspase-3 colorimetric assay kit (BioVision Research Products,USA).

the chromophore p-nitroanilide (pNA) after cleavage from the labeled substrate DEVD-pNA was measured, spectrophotometricly. After incubation of reaction mix and cells, the mixture was read under 405 nm wavelengths on an Elisa reader (Thermo Electron Corporation Multiskan Spectrum, Finland). The absorbance values are normalized to protein concentrations and determined using a Bradford assay.

Phosphatidylserine exposure and cell permeability

It is a well-known outcome that when the cell death occurs by apoptosis, phosphatidylserine (PS) components change its location through the cell surface. This information makes PS as an early apoptotic marker for cell death. For this purpose, the level of PS can be detected by staining with the green fluorescent dye Annexin V-FITC (BD Pharmingen, Germany).

After cells and dyes were suspended in 250 µl buffer and incubation, cells mixture were analyzed immediately using flow cytometry.

Statistical analysis

Results are expressed as the mean standard error of the mean (SEM). The data were analyzed using one-way ANOVA.

Results

Succinic acid and endometrium carcinoma cell line in a time- and dose-dependent manner

Due to the determination of antiproliferative effects for succinic acid on CRL-2923 and MRC-5 healthy cells, the cells were incubated with increasing concentrations (0,5, 1, 2,5, 5, 10, 25, 50, and 100 µM) of succinic acid for 24, 48, and 72 h using WST-1 cell proliferation assay. According to results of WST-1 assay, important changes was found decreasingly in cell proliferation level in the endometrium cancer cell line as compared with controls (*p<0.05) (Figure 1). Moreover, there were no important changes in the healthy cell line.

Succinic acid induces the loss of mitochondrial membrane potential

To determine the loss of MMP, CRL-2923 and MRC5 cells were exposed to 5 µM and 10 µM succinic acid for 48 h and JC-1 MMP assay was performed. The results of this assay showed that there was a 2.47-fold increase in loss of MMP in response to 5 µM succinic acid for 48 h CRL-2923 cells, as compared with untreated cells (*p<0.05)(Figure 2). There was no change in MMP potential of the MRC-5 cells under the same conditions (Figure 2).

Succinic acid decreases caspase-3 enzyme activity

The CRL-2923 and MRC-5 cells were incubated with increasing concentrations of succinic acid for 48h and changes in caspase-3 enzyme activities were analyzed to find the relation for apoptotic way. Compared with the untreated cells, there was a 0.36-fold decrease in caspase3 activity in response to 48h incubation with 5 µM, and a 0.18-fold decrease in response to 48h incubation with 10 µM on CRL-2923 cells (*p<0.05)(Figure 3). There was no change in caspase3 activity of MRC-5 cells under the same conditions (Figure 3). Succinic acid induced apoptosis in a dose-dependent manner, which could be related with caspase-7 activity.

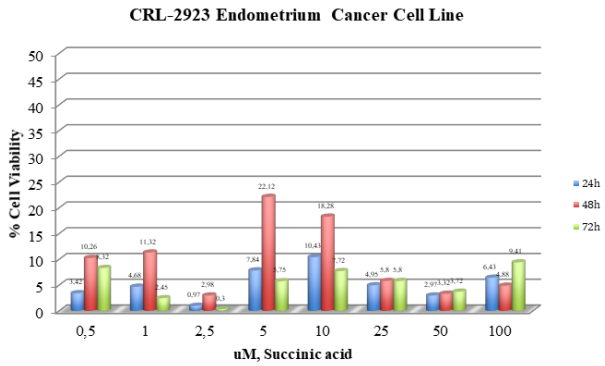

Figure 1. Succinic acid inhibited proliferation of endometrium carcinoma cell line in a time- and dose-dependent manner.

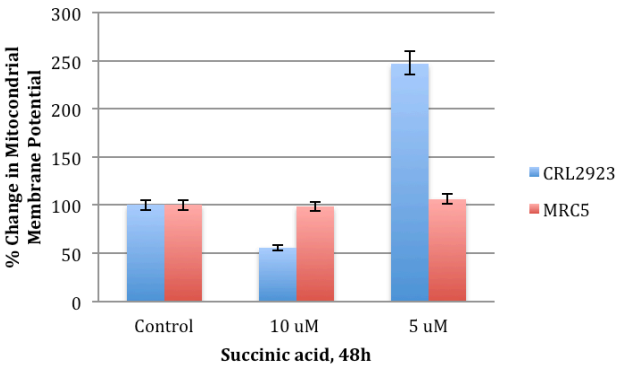

Figure 2. Succinic acid induces the loss of mitochondrial membrane potential on CRL-2923 and MRC5.

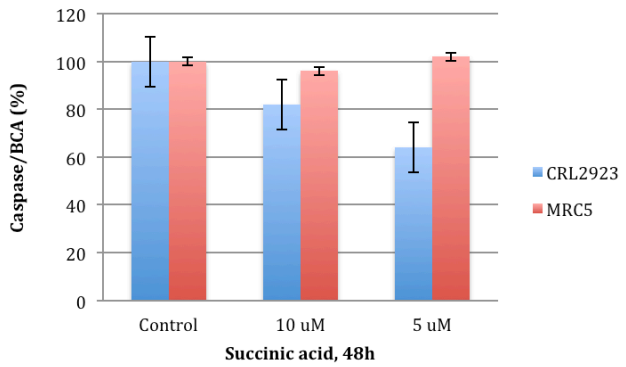

Figure 3. Succinic acid decreases caspase-3 enzyme activity on CRL-2923 and has no caspase-3 enzyme activity on MRC-5.

Succinic acid made the PS levels became available on the cell surface

FITC AnnexinV/PI double staining was performed in CRL-2923 endometrium cancer cell line and MRC-5 healthy lung fibroblastic cells, both of which had been exposed to 5 µM and 10 µM succinic acid for 48 hrs. The results demonstrated that 48hrs incubation of CRL-2923 cells with succinic acid increased apoptotic cell death when compared with the untreated control group (*p<0.05)(Figure 4). Furthermore, there were no changes in MRC-5 cell line.

Discussion

In this study, apoptotic and necrotic effects of succinic acid was determined for the first time in an endometrial cancer cell line. Succinic acid is accessible in daily bakery products, aromatized products, and in some vegetables (3-5).

According to our cytotoxicity test, 5 µM and 10 µM doses were the most effective doses and 48 hours was determined the optimum time. 5 µM effected 42.08%, 10 µM effected 68.65% increasingly on apoptotic way, respectively. This finding shows that our study produced desired cell death results for apoptosis.

Apoptosis is regulated by programmed genes and it has the responsibilty to protect the overall homeostasis of the organism, which comprises metabolic and physiologic reactions (7,8). Increasing in the number of apoptotic cells means that there can be sufficient apoptotic death to remove undesirable tissues and cells, as a control mechanism (9).

Our results suggest succinic acid has important potential for cancer because it was effective on endometrial cancer and also because it had no adverse or necrotic effect on healthy cells. Succinic acid has no adverse effects because it is metabolized by body cells and it has a natural role in the TCA cycle as a media component. The TCA cycle and its enzyme component have some crucial roles for basal cell metabolism (6). TH e TCA cycle and its enzyme system are conventionally known for their provision of energy, respiratory chain, and carbon source for metabolism. In addition to this, the TCA cycle is also a center for important signaling molecules and gene expression. In particular, the relationship between the TCA caycles and cells under pathologic conditions should be investigated as an important issue (10). Metabolic profiling of endometrial cancer cells showed higher rates of glycolysis and lower glucose oxidation than their nonmalignant counterparts. However, oxidative metabolism in general was not defective in endometrial cancer cells (11,12). Although succinic acid dehydrogenase expression and the succinic acid signaling pathway have been elucidated, Habano et. al. 2003 and Frederiksen et al. 2003 suggested that they play a role in the formation of the stomach and colon cancer (13,14).

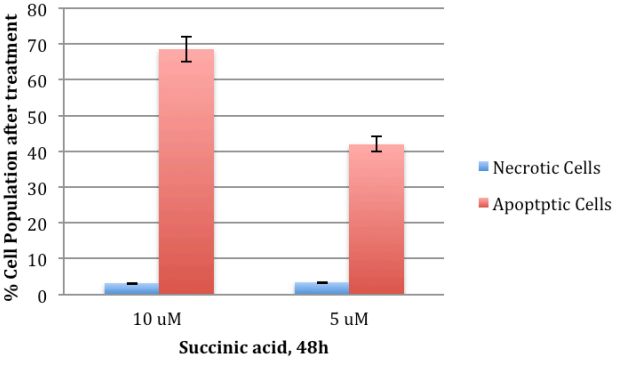

Figure 4. Succinic acid caused the apoptosis resulting in the translocation of PS on CRL2923.

As a result of succinic acid's apoptotic success in this study on endometrial cancer, succinic acid might be used as an anticancer treatment agent. This potential should be researched with further analysis to understand the underlying mechanism. It might be an important step in the development of a new targeted therapy in endometrial cancer.

Acknowledgements

Istanbul University Scientific Research Projects; 38644. We would like to thank to Mr. David F. Chapman for English editing.

Interest conflict

The authors declare that they have no financial disclosures or conflicts of interest.

References

1. Macmahon B. Risk factors for endometrial cancer. Gynecologic Oncology 1974; 2:122.
2. Modugno F, Ness RB, Chen C, Weiss NS. Inflammation and endometrial cancer: a hypothesis. Cancer epidemiology, biomarkers & prevention 2005; 14:2840-2847.
3. Lan L, Tong-Xun L, Mou-Ming Z, Chun C, Bo-En Y, Sheng T, Fei Y. Functional, nutritional and conformational changes from deamidation of wheat gluten with succinic acid and citric acid. Food Chemistry 2010; 123:123-130.
4. Vinay BJ, Sven C, Bert L, Kevin JV, Jan AD, Christophe MC. Mapping of Saccharomyces cerevisiae metabolites in fermenting wheat straight-dough reveals succinic acid as pH-determining factor. Food Chemistry 2013; 136:301–308.
5. Yong-lan X, Ke-quan C, Wen-yu D, Jiang-feng M, Min Z, Min J, Ping W, Ping-Kai O. Succinic acid production by Actinobacillus succinogenes NJ113 using corn steep liquor powder as nitrogen source, Bioresource Technology 2013; 136:775-779.
6. Akira T, Sanae O, Pascal H, Eiichi S. Metabolic engineering of Lactobacillus plantarum for succinic acid production through activation of the reductive branch of the tricarboxylic acid cycle. Enzyme and Microbial Technology 2013; 53:97-103.
7. Bellamy COC, Malcomson RDG, Harrison DJ, Wyllie AH. Cell

death in health and disease: the biology and regulation of apoptosis. Seminar in Cancer Biology 1995; 6:3-16.
8. Ellis RE, Yuan J, Horvitz HR. Mechanisms and functions of cell death. Annual Review of Cell and Developmental Biology 1991; 7:663-698.
9. Evan G, Littlewood T. A matter of life and cell death. Science 1998; 281:1317-1321.
10. Seyfried TN, Flores R, Poff AM, D'Agostino DP. Cancer as a metabolic disease: implications for novel therapeutics. Carcinogenesis 2009; 14:625–635.
11. Tyler D. The Mitochondrion in Health and Diseases. New York: Weinheim; 557; 1992.
12. Whelan DT, Hill RE, McClorry S. Fumaric aciduria: a new organic aciduria, associated with mental retardation and speech impairment. Clinica Chimica Acta 1983; 132:301–308.
13. Habano W, Sugai T, Nakamura S, Uesugi N, Higuchi T, Terashima M, Horiuchi S. Reduced expression and loss of heterozygosity activation of the of the SDHD gene in colorectal and gastric cancer. Oncology Report 2003; 10:1375–1380.
14. Frederiksen CM, Knudsen S, Laurberg S, Orntoft TF. Classification of Dukes' B and C colorectal cancers using expression arrays. Journal of Cancer Research and Clinical Oncology 2003; 129:263–271.