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The Effects of Rutaecarpine on Metastatic Prostate Cancer Cells

Mehmet Abdulkadir Şekeroğlu[1], Erdem Çokluk[1*], Zeynep Özman[2], Asuman Deveci Özkan[3], Gamze Guney Eskiler[3], Mehmet Ramazan Şekeroğlu[1], Fatıma Betül Tuncer[1]

[1]Department of Medical Biochemistry, Faculty of Medicine, Sakarya University, Sakarya, Turkey
[2]Department of Medical Biochemistry, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey
[3]Department of Medical Biology, Faculty of Medicine, Sakarya University, Sakarya, Turkey

ABSTRACT

Prostate cancer with an increased incidence in the world is one of the public health-threatening malignancy. Metastatic prostate cancer is an important cause of death in men despite of the combined use of more than one chemotherapeutic drug as well as radiotherapy and supportive treatments. Therefore, there is a need to develop novel treatment strategies in metastatic prostate cancer patients. The aim of this study was to investigate the potential therapeutic effects of Rutaecarpine (RUT) on metastatic prostate cancer cells. RUT induced cytotoxicity and apoptotic cell death were evaluated by WST-1, Annexin V, AO staining and ELISA assays in PC-3 human metastatic prostate cancer cell line.

The viability percentage of PC-3 cells after exposing to different concentrations of RUT treatment significantly decreased in a time and dose dependent manner and the most effective concentrations of RUT was determined as 20 and 40 μM for 48 hours ($p<0.05$). Annexin V and AO staining revealed that the early and late apoptosis rate significantly increased compared to the control group ($p <0.05$). Additionally, the caspase-3 levels significantly increased after RUT treatment in PC-3 cells in a dose dependent manner ($p<0.05$). In this study, RUT exhibited a cytotoxic and apoptotic effects on PC-3 cells and therefore RUT could be a potential new therapeutic agent for the treatment of metastatic prostate cancer. However, the underlying mechanism of the apoptotic death caused by RUT in PC-3 cells should be further investigated through advanced analysis at molecular level.

Keywords: Apoptosis, Metastatic prostate cancer, Rutaecarpine, Caspase-3

Introduction

Prostate cancer is one of the malignancies and its incidence is growing worldwide especially with the increase of the elderly population (1,2). It is the second most common cancer type in men (3). During the treatment and follow-up process of prostate cancer, risk assessment is evaluated according to the TNM stage, Gleason score, histopathological character, and serum prostate specific antigen levels (4). Surgical therapy (radical prostatectomy), radiotherapy (RT) and hormonal therapy (HT) approaches are used alone or in combination in prostate cancer according to the risk groups. However, conventional methods in metastatic prostate cancer do not seem as successful as in other malignancies (5, 6). Furthermore, the treatment of metastatic prostate cancer remains the most significant challenge (7). Therefore, there is an urgent need to develop more effective novel treatment strategies in metastatic prostate cancer patients.

Rutaecarpine (RUT) is an alkaloid compound obtained from a traditional Chinese plant called Evodia rutaecarpa (8). This compound, which has been used for thousands of years to treat various diseases such as gastrointestinal diseases, amenorrhea, and postpartum bleeding, exerts many biological effects, including anti-inflammatory, anti-obesity, and anti-tumor activities (9-13). The anti-cancer efficacy of RUT is based on the inhibition of topoisomerase I and topoisomerase II in cancer cells (11). Topoisomerase inhibitors are antineoplastic agents commonly used in clinical practice alone or in combination with other chemotherapeutic drugs that inhibit a group of key enzymes in cancer. These agents create cytotoxic effects by interfering with the normal function of the cell during DNA replication and transcription and have an important role in the treatment of

*Corresponding Author: Erdem Çokluk, Medical Faculty, Sakarya University Adapazarı Sakarya
E-mail: erdemcokluk205@hotmail.com, Phone: 0 (506) 4971615, Fax: 0 (264) 295 66 29
ORCID ID: Mehmet Abdulkadir Şekeroğlu: 0000-0001-5530-8684, **Erdem Çokluk:** 0000-0002-6205-5109, **Zeynep Özman:** 0000-0002-8415-6883, **Asuman Deveci Özkan:** 0000-0002-3248-4279, **Gamze Guney Eskiler:** 0000-0002-2088-9914, **Mehmet Ramazan Şekeroğlu:** 0000-0001-8383-6740, **Fatıma Betül Tuncer:** 0000-0002-4034-4188
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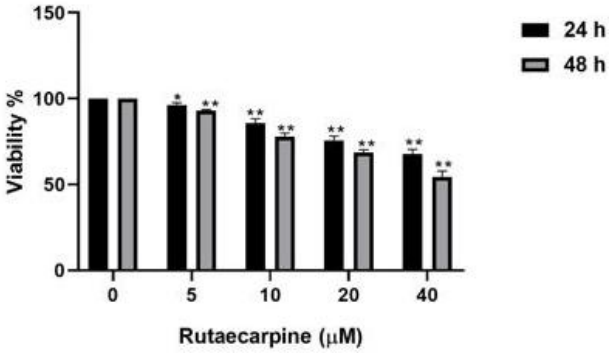

Fig. 1. The viability rates of PC-3 cells following incubation with different concentrations of RUT (5, 10, 20 and 40 μM) for 24 and 48 h compared with control group (*p<0.05, **p<0.01)

neoplastic diseases (11, 14). In prostate cancer, it has been reported that evodiamine, a structural analogue of RUT, inhibits the cell cycle and the growth of prostate cancer cell lines through the induction of apoptosis (15). However, the potential therapeutic effects of RUT on metastatic prostate cancer cells have not been investigated. In this study, we aimed to investigate the cytotoxic and apoptotic effects of RUT on metastatic prostate cancer cells.

Materials and Methods

Cell Culture and RUT Treatment: The human metastatic prostate cancer cell line (PC-3) was commercially obtained from the American Type Culture Collection (ATCC). The cells were cultured in Roswell Park Memorial Institute 1640 medium (RPMI 1640, Gibco) supplemented with 10% Fetal Bovine serum (FBS) and incubated at 37 °C with 5% CO2. The active powder form of RUT (Sigma-Aldrich) was purchased commercially and dissolved in 0.1% Dimethyl Sulfoxide (DMSO) in sterile conditions and the main RUT stock solution was prepared. RUT was prepared from the main stock solution (in concentrations of 5, 10, 20, 40 μM) by dilution with the medium.

WST-1 Analysis: PC-3 cells were seeded in 96-well cell culture plates at 2x104 cells/well and incubated with different concentrations of RUT (5, 10, 20 and 40 μM) at 37 ° C for 24 and 48 h. After incubation, the cells were incubated with 10 μL of WST-1 reagent (Biovision) in the dark for 45 min at 37 ° C and analyzed with an Enzyme-Linked ImmunoSorbent Assay (ELISA)-reader (Allsheng, China) in the 460-620 nm wavelength range. The most effective concentrations and exposure time were selected for further experiments.

Annexin V Analysis: After incubation with RUT (20 and 40 μM) for 48 h, the cells were stained with Muse Annexin V & Dead Cell Assay kit (Millipore). Finally, the percentage of apoptotic and/or necrotic cells was determined using the Muse Cell Analyzer (Millipore, Germany).

Acridine Orange Staining: To examine whether RUT caused a morphological change in PC-3 cells, the cells were seeded in 6-well cell culture (5x105 cells/well) and treated with 20 and 40 μM RUT for 48 h. After incubation, the cells were fixed with 4% cold-paraformaldehyde and stained with AO (100 mg/ml) for 30 min in the dark. Finally, the cells were analyzed with the EVOS Cell Imaging System (Thermo Fisher Scientific, USA).

ELISA Analysis: For caspase-3 analysis, the cells were seeded in 6-well cell culture plates and incubated with 20 and 40 μM of RUT for 48 h. After incubation, caspase-3 level was measured with the Caspase 3 Human Instant ELISA Kit (Thermo Fisher Scientific) according to the kit protocol and at analyzed in 450 nm wavelength in ELISA reader (Allsheng, China).

Statistical analysis: GraphPad Prism 6.0 program was used for statistical analysis, and the differences between groups were evaluated by One Way ANOVA (Post-Tukey) analysis. p <0.05 values were accepted as statistically significant.

Results

Evaluation of the Cytotoxic Effect of RUT on PC-3 Cells: The cytotoxic effects of RUT on PC-3 cells were determined by WST-1 assay and the obtained results were given in Figure 1. The viability rate of PC-3 cells significantly decreased to 96.43% ± 0.65%, 85.86% ± 1.83%, 75.70± 0.51% and 67.96 ± 0.92% at 5, 10, 20 and 40 μM, respectively for 48h (Figure 1, p<0.05). According to the results, the most effective exposure concentrations (20 and 40 μM) of RUT and time (48 h) were determined in PC-3 cells through WST-1 assay.

Evaluation of the Apoptotic Effect of RUT on PC-3 Cells: The apoptotic effects of RUT on the PC-3 cells were determined by Annexin V analysis and the obtained results were presented in Figure 2. The number of early apoptotic cells for 48 h treatment were significantly increased in a dose dependent manner (p<0.01, Figure 2A). After 48 h incubation of 20 μM RUT, the proportion of early and late apoptotic cells was 21.49% ± 0.87 and 9.69% ± 0.46, respectively. However, 40 μM RUT treatment resulted in a significant increase in

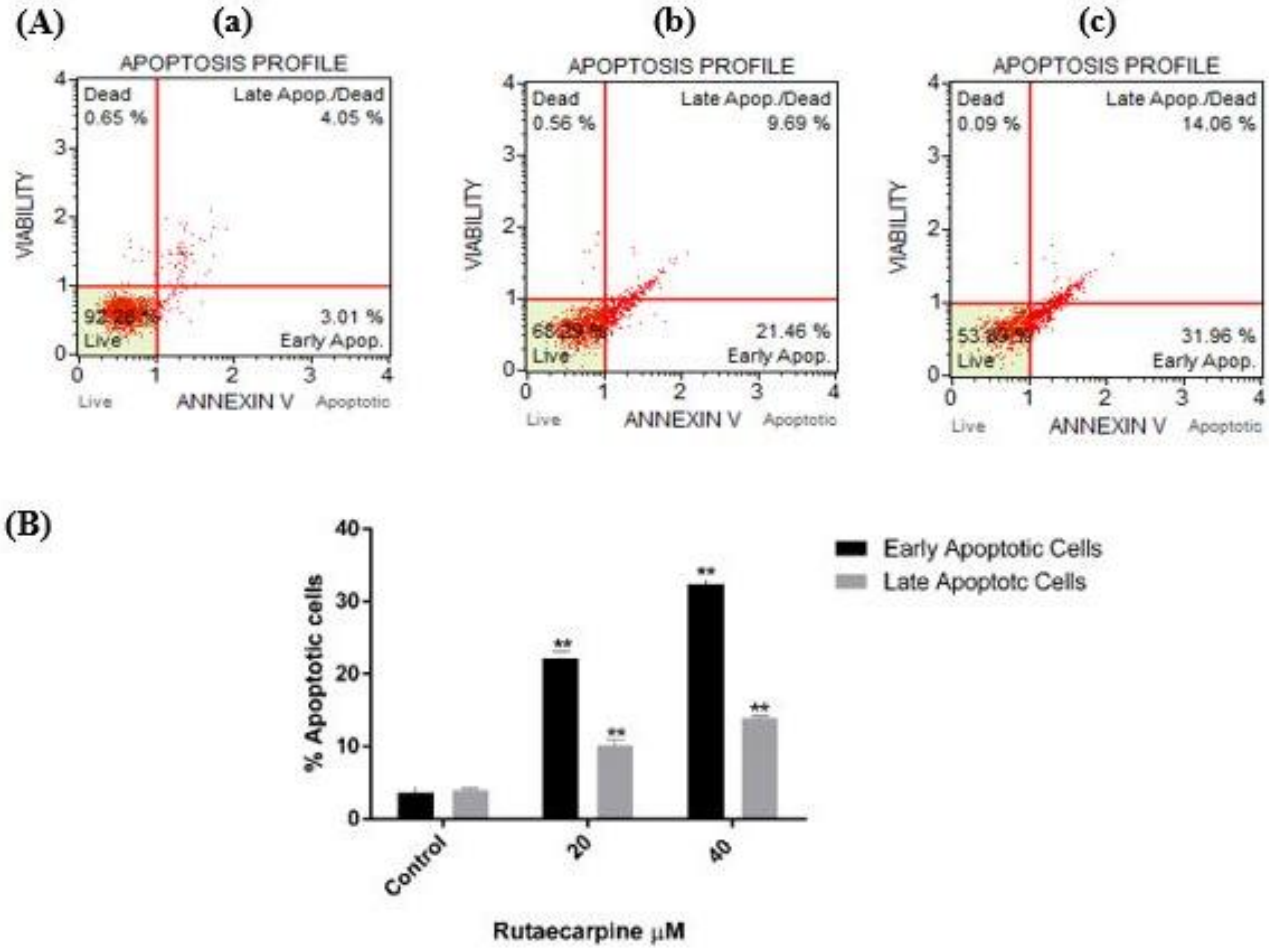

Fig. 2. Determination of the apoptotic effect of RUT on PC-3 cells by Annexin V analysis. (A) (a) Control, (b) 20 and (c) 40 μM RUT treatment for 48 h, B) Statistical comparison of the percentage of early and late apoptotic cells compared with the control group (* $p < 0.05$, ** $p < 0.01$)

the proportion of early and late apoptotic cells (31.96 ± 1.65% and 14.06 ± 1.54%, respectively) (Figure 2B, $p < 0.01$).

Evaluation of the Morphological Changes in PC-3 Cells: The effects of RUT on PC-3 cell morphology determined by AO staining were shown in Figure 3. According to the observations with fluorescent microscope, decreased nuclear cytoplasmic ratio and differences in general cell morphology were observed after treated with RUT (20 and 40 μM) for 48h in PC-3 cells. Therefore, RUT treatment caused apoptotic cell morphology in a dose dependent manner and the obtained results were consistent with WST-1 and Annexin V results.

Evaluation of the Caspase-3 Level in PC-3 Cells: RUT induced caspase-3 level in PC-3 cells was determined by ELISA analysis. Caspase-3 protein levels considerably increased following treatment with RUT in a dose dependent manner in PC-3 cells compared to the control group (Figure 4, $p<0.01$). After 48 h incubation of 20 and 40 μM RUT, the caspase-3 protein levels were 0.461 and 0.651 ng/ml compared with the control group (0.105 ng/ml), respectively. (Figure 4, $p < 0.01$).

Discussion

In the present study, our findings showed that RUT exerted cytotoxicity and induced apoptotic cell death through increased caspase-3 level in PC-3 cells in dose and time dependently.

RUT is an alkaloid with different biological effects such as, anti-inflammatory, anti-obesity, and anti-tumor (9-13, 16). Therefore, the anti-proliferative effects of RUT and its potential as a chemotherapeutic agent have been investigated in different cancer types. Ming et al. (17) state that RUT has a cytotoxic effect on the colon, breast, and liver cancer, in vitro. Additionally, evodiamine (EVO) and RUT derivatives have been found as cytotoxic in HeLa and PC-3 cells (18, 19). However, the efficacy of chemical modification of their derivatives has been evaluated in this study.

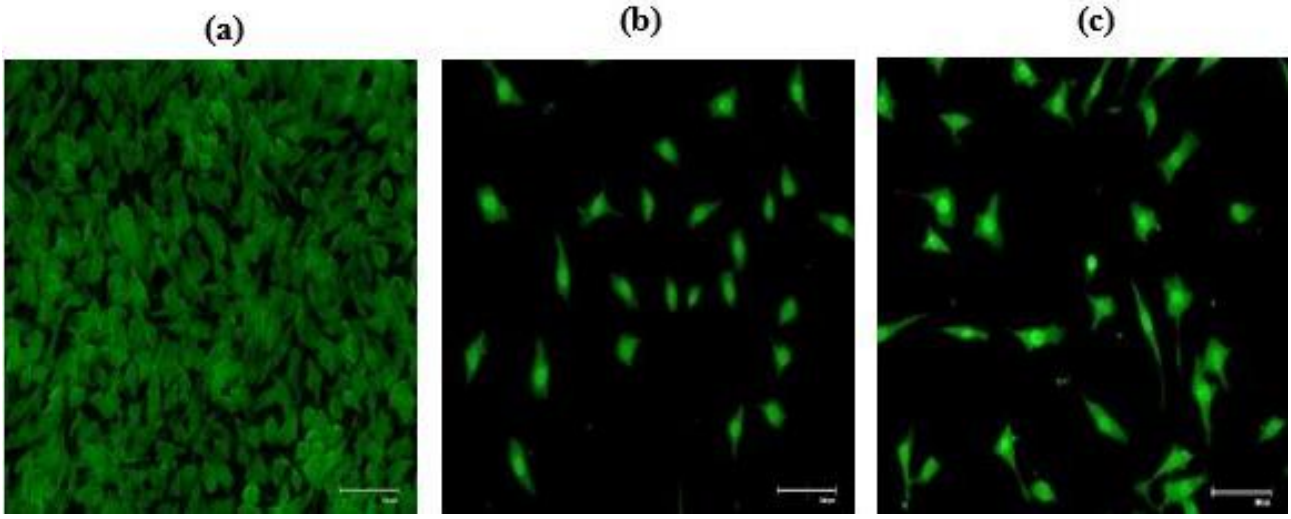

Fig 3. Determination of the effect of RUT on cell morphology by AO staining. Morphological changes in (a) Control, (b) 20 and (c) 40 μM RUT treated PC-3 cells for 48 h

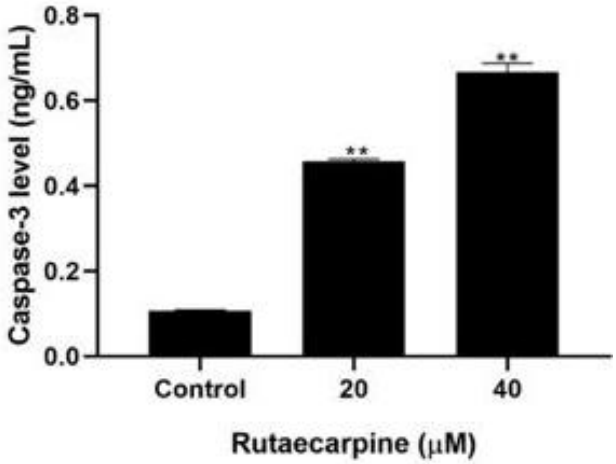

Fig. 4. The effect of RUT on caspase-3 levels in PC-3 cells. Comparison of different concentrations of RUT (20 and 40 μM) in PC-3 cells with the control group (** $p < 0.01$) for 48 h

Therefore, the therapeutic effects of RUT on metastatic prostate cancer have not been studied. In our study, the potential cytotoxic effect of RUT on PC-3 cells was investigated by WST-1 analysis and the viability rate of the PC-3 cells significantly decreased compared to the control group in a dose and time dependent manner ($p<0.05$).

Furthermore, the apoptotic cell death induced by RUT and/ EVO have been evaluated in different cancer cells. Ching et al. (20) have examined the cytotoxic and apoptotic effects of evodiamine (EVO) and RUT on the SKOV3 ovarian cancer cells. They have found that both EVO and RUT exert cytotoxic effects and induce apoptosis in SKOV3 cells through G2/M arrest. Zhang et al. (21) show that RUT treatment strongly induces apoptosis by activating caspase-3 in the gastric cancer SGC-7901 cell line. In consistent with the literature, in our study, Annexin V analysis demonstrated that the apoptotic effect of RUT on PC-3 cells significantly increased in a dose dependent manner through the activation of caspase-3 level.

In conclusion, RUT caused a cytotoxic and apoptotic effects on PC-3 metastatic prostate cancer cells. However, the molecular mechanism of the apoptotic death caused by RUT in metastatic prostate cancer should be investigated in detail by in vitro and in vivo experiments. Additionally, the combine effects of RUT with different chemotherapeutic drugs on the treatment of metastatic prostate cancer can contribute to the literature.

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