



The Role of Steroid Receptors in the Proliferation and Migration of Endometrial Adenocarcinoma HEC1A Cell Line

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Abstract

Objectives: Endometrial carcinoma is one of the most common gynecological cancers. It is generally divided into oestrogen-dependent type I, and oestrogen-independent type II. Although the expression of some steroid receptors has been documented in type II endometrial carcinoma, their roles in tumor progression have not been fully elucidated yet. Thus in this study, we aimed to examine the role of compounds acting on steroid receptors in type II, on HEC1A cultured cells.

Methods: We tested the effect of mifepristone (the glucocorticoid and progesterone receptor blocker, 10-8M), bicalutamide (the androgen receptor blocker, 10-6M), G15 (the G-protein-coupled estrogen receptor-1 blocker, 10-7M) and PHTPP (2-Phenyl-3-(4-hydroxyphenyl)-5,7-bis (trifluoromethyl)-pyrazolo [1,5-a]pyrimidine, the estrogen receptor- β blocker, 10-7M), on proliferation. Proliferation was assessed by xCELLigence analysis system and migration was examined by using wound-healing model.

Results: None of the drugs, at the used concentrations, have affected the proliferation of HEC1A cells. However, migration was significantly increased at the 24th and the 48th hour of mifepristone application ($p<0.05$, $P<0.01$, respectively). Bicalutamide also increased migration at the 24th hour ($p<0.05$). G15 and PHTPP did not change the migration of cells.

Conclusions: These results suggest that, unlike other steroid receptors, glucocorticoid and/or progesterone receptors may play an important role in the reducing migration of endometrial carcinoma and might be used as targets to reduce the metastasis of this type of cancer.

Keywords: Endometrial adenocarcinoma; steroid receptors; proliferation; migration

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Steroid Reseptörlerinin Endometriyal Adenokarsinoma HEC1A Hücre Hattının Proliferasyon ve Migrasyonundaki Rolü

Öz

Amaç: Endometriyal karsinoma en sık görülen jinekolojik kanserlerden biridir. Genellikle östrojene bağımlı olan tip I ve östrojenden bağımsız tip II olmak üzere iki gruba ayrılır. Bazı steroid reseptörlerinin ekspresyonu tip II endometriyal karsinomada gösterilmiş olmasına rağmen, tümör progresyonundaki rolleri henüz tam olarak aydınlatılamamıştır. Bu nedenle bu çalışmada, tip II, HEC1A kültüre edilmiş hücrelerde, steroid reseptörleri üzerinden etki gösteren bileşiklerin rolünü incelemeyi amaçladık.

Yöntemler: Mifepriston (glukokortikoid ve progesteron reseptör blokleri, 10-8M), bicalutamid (androjen reseptör blokleri, 10-6M), G15 (G-protein-bağlı östrojen reseptörü-1 blokleri, 10-7M), PHTPP' nin (2-Fenil-3-(4-hidroksifenil)-5,7-bis(triflorometil)-pirazolo[1,5-a]pirimidin, östrojen reseptörü-β blokleri, 10-7M), hücrelerin proliferasyon ve migrasyonu üzerine etkilerini test ettik. Proliferasyon, xCELLigence hücre analizi sistemi ve migrasyon, yara iyileştirme modeli ile değerlendirildi.

Bulgular: Kullanılan konsantrasyonlarda hiçbir ajan HEC1A hücrelerinin proliferasyonunu etkilememiştir. Ancak mifepriston uygulamasının 24. ve 48. saatlerinde migrasyon anlamlı olarak arttı ($p<0.05$, $P<0.01$). Bicalutamid uygulandıktan sonra 24. saatte migrasyonu arttırdı ($p<0.05$). G15 ve PHPP, hücrelerin migrasyonunu değiştirmedir.

Sonuç: Bu sonuçlar, diğer steroid reseptörlerinden farklı olarak, glukokortikoid ve/veya progesteron reseptörlerinin endometriyal karsinomun migrasyonunu azaltmada önemli bir rol oynayabileceğini ve bu kanser tipinin metastazını azaltmak için hedef olarak kullanılabileceğini düşündürmektedir.

Anahtar kelimeler: Endometriyal adenokarsinoma; steroid reseptörleri; proliferasyon; migrasyon.

INTRODUCTION

In developed countries, endometrial cancer is the most common gynecological cancer for women¹. Recently, both incidence and mortality related to this type of cancer have grown, with over 5918 new cases in Turkey in 2020². It is generally divided into oestrogen-dependent type I, and oestrogen-independent type II which accounts for 80-90% and 10-20% of all endometrial cancer cases respectively³. Although it is less common, approximately half of all endometrial cancer-related deaths are tied to type II due to its aggressive nature and resistance to current treatments⁴. Although type II endometrial cancer is typically viewed as not reliant on estrogen, research has suggested that both types of endometrial cancer share the same risk factors that are related to high estrogen or low progesterone levels. These risk factors encompass obesity, hormone replacement therapy, never having had a child, and estrogen-producing ovarian tumors, in addition to medical conditions and medication

that result in elevated estrogen levels, such as polycystic ovary syndrome. Hence, type II endometrial cancer may not be completely hormone-independent^{3,4}.

Steroid hormone receptors are intracellular transcription factors that are activated by particular ligands and control the activity of thousands of genes. Genes regulated by steroid hormone receptors are essential for many biological activities including cell proliferation, survival, metabolism, and differentiation^{5,6}. Therefore, impaired functioning of steroid receptors could lead to multiple forms of cancers such as breast, leukaemia and lymphoma, prostate, ovarian, and lung cancer⁶. Type II endometrial cancer is not dependent on oestrogen, some studies have highlighted the presence of oestrogen and progesterone receptor in many type II endometrial cancer cells⁷. Steroid receptors such as ERβ, GPER, PR, and androgen receptors have been detected in

Human Endometrial Cancer HEC1A cells, which demonstrate a papillary endometrial adenocarcinoma model^{8,9}. Nevertheless, the contribution of steroid receptors to type II endometrial cancer cells is yet to be completely understood.

The aim of the present study was to investigate whether steroid receptors have an influence on proliferation and migration of endometrial adenocarcinoma cells.

METHODS

Cell culture

Commercially available cancer cell line HEC1A (ATCC® HTB-112TM) which is human endometrial origin was obtained from ATCC (Manassas, VA, USA). McCoy's 5A-modified medium was supplemented with 10% fetal bovine serum (FBS), 2% penicillin/streptomycin and L-glutamine (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) and incubated at 37°C in a humidified atmosphere with 5% CO₂. Cells were subcultured every 7-10 days by sterile and standard techniques. Since the present work was conducted by a commercially available cell culture line; according to ethical rules in Turkey, there is no need for ethical approval.

Drugs preparation and experimental groups design

Mifepristone, bicalutamide, G15, PHTPP were purchased from Sigma Aldrich (St. Louis MO, USA). 0.05% ethanol used as a solvent for mifepristone and bicalutamide while 0.05% DMSO for G15 and PHTPP.

Data obtained from all experiments were compared with vehicle controls (0.05% ethanol or DMSO).

Proliferation assays

Proliferation assays were carried out by using a real-time cell analyzer xCELLigence device

(ACEA Biosciences Inc., California, USA). The device was placed in an incubator gassed with 5% CO₂ at 37°C using 16-well E-plates (ACEA Biosciences Inc., California, USA). The experiment was done according to the software manual as described previously¹⁰.

Migration Assays

Migration was conducted by the wound-healing model. Each well of 24-well plates (Costar®; Sigma Aldrich, St. Louis MO, USA) was seeded with 30000 cells. The procedure and calculations were done as described previously (19). Briefly after the confluency the cells were incubated for 24 hours with serum free medium. Wound lines were opened by using a 20 µl pipette. Thereafter 1% FBS containing medium was added to the wells. After the agents' administration (t = 0) at 24th and 48th hours, the wound images were recorded. Relative cell motility was generated by the equation (wound width at t = 0 hours) - (wound width at t = 24 or 48 hours).

Statistical Analysis

Alterations in cellular multiplication were obtained from electrical impediments in the cell analyzer and expressed as cell index "CI". A CI value, greater than 0.2 was regarded as positive, and data were represented as mean ± SD but mean ± SEM for migration. One-way analysis of variance and Bonferroni post hoc test or t-test were utilized when necessary. *P*-values of less than 0.05 were regarded as significant.

RESULTS

Steroid receptors blockers in proliferation

The effect of 10⁻⁸M mifepristone on proliferation

The glucocorticoid and progesterone receptor blocker, mifepristone did not change cell proliferation at 10⁻⁸M compared to the ethanol group (Fig. 1, *p* > 0.05, *n*=6).

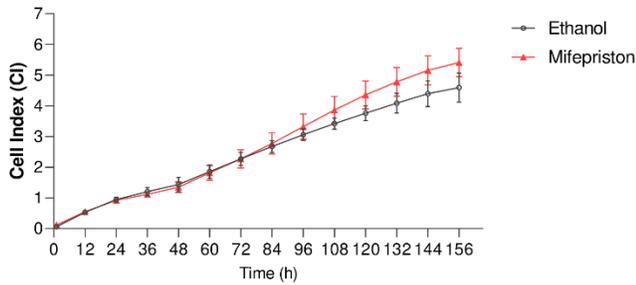


Figure 1: The effect of 10^{-8} mifepristone on the proliferation of HEC1A cells.

The effect of 10^{-6} M bicalutamide on proliferation

The androgen receptor blocker, bicalutamide did not change cell proliferation at 10^{-6} M when compared with the ethanol group (Fig. 2, $p > 0.05$, $n=5$).

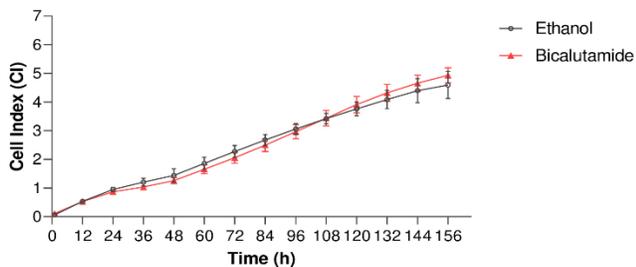


Figure 2: The effect of 10^{-6} bicalutamide on the proliferation of HEC1A cells.

The effect of 10^{-7} M G15 on proliferation

GPER-1 blocker, G15 did not change cell proliferation at 10^{-7} M when compared to the ethanol group (Fig. 3, $p > 0.05$, $n=6$).

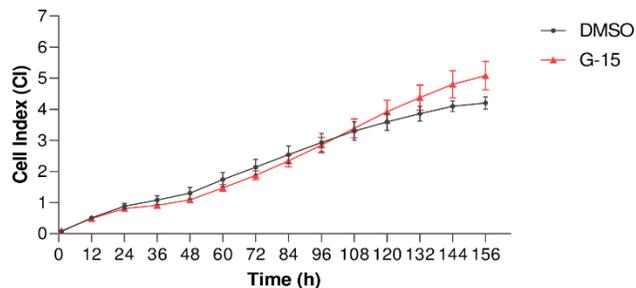


Figure 3: The effect of 10^{-7} G-15 on the proliferation of HEC1A cells.

The effect of 10^{-7} M PHTPP on proliferation

The oestrogen receptor- β blocker, PHTPP did not change cell proliferation at 10^{-7} M when

compared to the ethanol group (Fig. 4, $p > 0.05$, $n=6$).

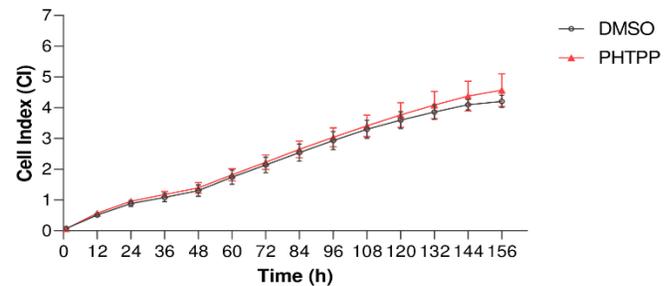


Figure 4: The effect of 10^{-7} PHTPP on the proliferation of HEC1A cells.

Steroid receptors blockers in migration

The effect of 10^{-8} M mifepristone on migration

At a concentration of 10^{-8} M, mifepristone significantly increased relative cell motility at both 24 and 48 hours when compared to the ethanol group (Fig. 5, $p < 0.01$, $n=4$).

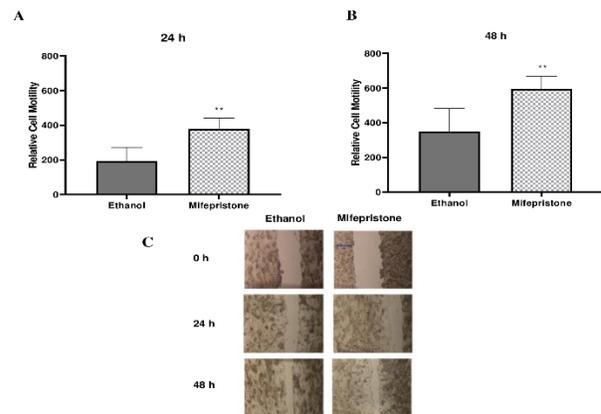


Figure 5: The effect of 10^{-8} mifepristone on the migration of HEC1A. (A) 24 hours and (B) 48 hours. (C) Representative wound figures at 0, 24 and 48 hours.

The effect of 10^{-6} M bicalutamide on migration

Bicalutamide (10^{-6} M) significantly increased cell migration at 24 hours ($p < 0.05$). However, this effect was appeared to be statistically insignificant at 48 hours (Fig. 6, $p > 0.05$, $n=4$).

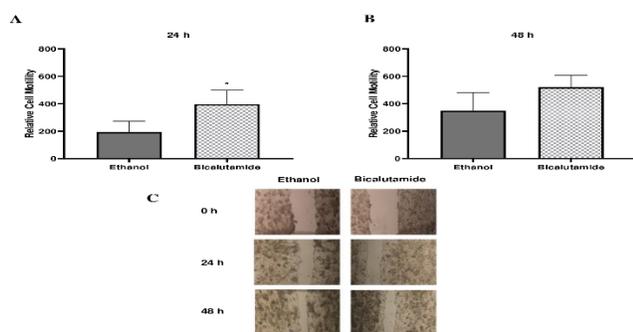


Figure 6: The effect of 10-6 bicalutamide on the migration of HEC1A. (A) 24 hours and (B) 48 hours. (C) Representative wound figures at 0, 24 and 48 hours.

The effect of 10-7M G15 on migration

G15 did not show any effect on cell migration at any time point when compared to the DMSO group (Fig. 7, $p > 0.05$, $n=4$).

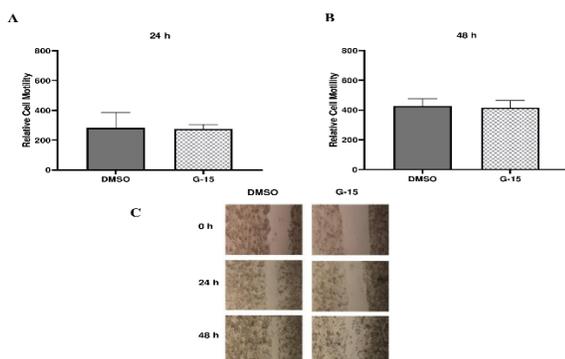


Figure 7: The effect of 10-7 G-15 on the migration of HEC1A. (A) 24 hours and (B) 48 hours. (C) Representative wound figures at 0, 24 and 48 hours.

The effect of 10-7M PHTPP on migration

PHTPP did not show any effect on cell migration at any time point when compared to the DMSO vehicle group (Fig. 8, $p > 0.05$, $n=4$).

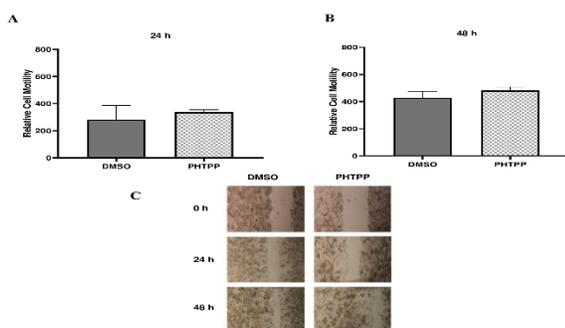


Figure 8: The effect of 10-7 PHTPP on the migration of HEC1A. (A) 24 hours and (B) 48 hours. (C) Representative wound figures at 0, 24 and 48 hours.

DISCUSSION

In the present study, the roles of compounds acting on steroid receptors on the proliferation and migration of human endometrial HEC1A cell line were investigated. For this purpose, we used four different steroid receptor blockers: mifepristone (10^{-8}M), bicalutamide (10^{-6}M), G15 (10^{-7}M) and PHTPP (10^{-7}M). Mifepristone and bicalutamide increased migration in endometrial adenocarcinoma HEC1A cells, but did not affect proliferation. However, the effect of bicalutamide on migration disappeared at the 48th hour. The other drugs change neither proliferation nor migration.

Many studies investigated the effect of mifepristone on different human cancer cells, including endometrial cancer. Aimin et al., showed that mifepristone at 10 μM and 100 μM concentrations reduced the proliferation of type I endometrial cancer Ishikawa cells¹¹. A similar study showed that 50 to 100 μM mifepristone reduced the proliferation and wound healing rate of HUUA endometrial cancer cells¹². However, the concentrations used in these studies were very high and cannot be used to predict the effect in human cancer tissue. It has been proven that tissue concentration of mifepristone is lower or equal to serum and the serum concentration does not increase with the increasing dose of mifepristone and does not exceed 2.5 μM ¹³. Consistent with our results, an in vitro study showed that clinically reasonable doses (0.1-5 μM) of mifepristone significantly increased the proliferation of ovarian cancer KK-1 cells, and only higher concentrations (7.5-25 μM) achieved an in vitro antitumor effect¹³. In addition, the use of mifepristone in the treatment of many cancers has failed in human clinical trials. A phase II clinical trial showed that treatment with mifepristone resulted in stable disease in only 25% of progesterone receptor-positive endometrial cancer patients and 75% of patients experienced disease progression.

The androgen receptor antagonist, bicalutamide, has been clinically utilized in combination with other drugs to treat metastatic prostate cancer¹⁴. Studies by bicalutamide in many cancers in vitro used concentrations ranging from 0.1 μ M to 100 μ M¹⁵⁻¹⁸. It has been reported that bicalutamide inhibits the growth of LNCaP prostate cancer, reduces their proliferation and migration, and induces apoptosis in a concentration-dependent manner¹⁵⁻¹⁸. In 2017, Robinson et al. demonstrated that the growth of triple-negative breast cancer cells was drastically decreased when exposed to 1 μ M bicalutamide¹⁹. A more recent study showed that doses of 25 μ M and 100 μ M of bicalutamide caused a dose-dependent reduction in the proliferation, invasion and migration of MDA-MB-231 cells²⁰. Hao et al. tested bicalutamide (50 μ M) on bladder cancer UM-UC-3 cells in 2017 and found that it induced cell apoptosis²¹. In 2021, a study revealed that 40 μ M of bicalutamide triggered apoptosis and inhibited the proliferation of gastric cancer cells²². Moreover, dihydrotestosterone was demonstrated to inhibit estradiol-induced proliferation of HEC1B endometrial cancer cells, and this inhibitory effect was significantly inhibited by co-administration of 1 μ M bicalutamide⁹. In our experiment, when 1 μ M bicalutamide was administered on its own, without dihydrotestosterone or estradiol, it had impact neither on the proliferation nor on the migration of HEC1A endometrial cells. These outcomes may suggest that a low concentration of bicalutamide can only block androgen receptors without influencing the proliferation and migration of endometrial cancer cells.

Since the GPER antagonist, G15, has been identified recently, the literature is scarce of studies testing its effects on cancer cells. It was reported that G15 alone had no effect on JKT-1 testicular cancer cell proliferation but it completely abolished their estradiol-induced

proliferation²³. Lio et al demonstrated that at concentrations of 0.1, 1, and 10 μ M, G15 inhibited the estradiol-induced proliferation of H1793 non-small cell lung cancer. They did not test G15 alone to check its effect²⁴. When applied to breast cancer cells, G15 did not have cytotoxic effects at levels lower than 0.625 μ M, but it significantly reduced their viability when applied at higher concentrations (1.25, 2.5 and 5 μ M)²⁵. Luo et al. also found that the application of 1 μ M G15, abolished the proliferative effect of estradiol on breast cancer-associated fibroblasts²⁶. At the same time, Bustos et al. have also demonstrated a significant reduction of MCF-7 breast cancer cell invasion with the application of G15²⁷. We did not examine the effect of G15 in combination with estradiol. Consistent with the literature, G15 alone did not affect cell proliferation and migration in HEC1A cells.

PHTPP has an ER β antagonistic activity with 36 times more selective on ER β than ER α ²⁸. Therefore, PHTPP has been used to test the role of ER β in normal and cancer cells. In 2013, Hsu and colleagues observed that 10 and 20 μ M PHTPP decreased the growth and invasion of breast cancer J82, 647v and T24 cells²⁹. Another study showed that the proliferation of breast cancer cells was significantly reduced by applying 1 μ M PHTPP³⁰. Mendes and colleagues also found that 50 μ M PHTPP caused a significant increase in MDA-MB-231 and MCF7 cells apoptosis³¹. However, PHTPP was shown to inhibit the cell proliferation of esophageal cancer by concentration-dependent manner³². In contrast, studies using PHTPP at lower concentrations have shown opposite results. A study conducted on ovarian cancer cells found that administration of 100 and 1000 pM PHTPP increased the proliferation of SCOV3 cells and administration of an ER β agonist significantly reduced their proliferation³³. Similarly, 1 and 10 nM PHTPP enhanced the proliferation of type I endometrial cancer and temporarily increased

the proliferation of HEC1A cells on the third day of administration³⁴. In our study, 10⁻⁷ M PHTPP (100 nM) did not show any effects on type II endometrial cancer HEC1A cells in terms of both proliferation and migration. This might be due to the difference in cancer type, the concentration used and other experimental procedures³⁵.

CONCLUSION

In the study, we used a single concentration of each drug to test the effects of steroid receptors. These results need to be checked with a range of concentrations for each drug. In conclusion, progesterone, glucocorticoid and androgen receptors appear to be important targets for inhibiting migration in endometrial adenocarcinoma HEC1A cells. However, more detailed studies are needed to make a decisive conclusion.

Ethics Committee Approval: This study was not conducted with human participants or animals.

Conflict of interest: The authors declare that there is no conflict of interest

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