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Türk Hijyen ve Deneysel Biyoloji Dergisi

N-acetyl cysteine decreases migratory capacity and increases endoplasmic reticulum stress in endometriotic cells with time and dose-dependent use

N-asetil sistein, zamana ve doza bağlı kullanım ile endometriyotik hücrelerde migrasyon kapasitesini azaltır ve endoplazmik retikulum stresini artırır

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ABSTRACT

Objective: Endometriosis is a chronic condition that affects women of reproductive age and is linked to infertility and pelvic pain. There hasn't been a curative treatment for the condition either, only anti-inflammatory drugs, analgesic drugs, and laparoscopy. An anti-oxidant N-acetylcysteine (NAC) restores glutathione (GSH). N-acetylcysteine has been used to treat endometriosis both in vivo and in vitro in a small number of trials. In vivo and in vitro studies have shown that NAC lowers inflammation and ectopic endometrial development. In this study, we aimed to decrease the proliferative capacity as well as the migratory behavior of the endometriotic cells by using the IC50 level of NAC.

Methods: HESC and 12Z human endometrial and endometriotic stromal cell lines were expansed according to the manufacturer's instructions. Impedance measurements were performed on the xCELLigence (RTCA) device to determine cellular impedance. N-acetyl cysteine was applied to the cells at doses between 10^{-6}-10^{-12} M, and the IC50 for NAC was calculated. The cells were treated with IC50 NAC, and the wound healing

ÖZET

Amaç: Endometriyozis üreme çağındaki kadınları etkileyen ve kısırlık ve pelvik ağrı ile bağlantılı kronik bir hastalıktır. Hastalık için henüz iyileştirici bir tedavi olmayıp, sadece laparoskopi yapılmakta, anti-inflamatuar ilaçlar ve analjezik ilaçlar verilmektedir. Bir antioksidan olan N-asetilsistein (NAC), glutatyonu (GSH) geri kazandırır. N-asetilsistein, az sayıda çalışmada hem in vivo hem de in vitro endometriyozisi tedavi etmek için kullanılmıştır. In vivo ve in vitro çalışmalar, NAC'nin inflamasyonu ve ektopik endometriyal gelişimi azalttığını göstermiştir. Bu çalışmada, NAC'nin IC50 seviyesini kullanarak endometriyotik hücrelerin proliferatif kapasitesinin yanı sıra migrasyon davranışını da azaltmayı amaçladık.

Yöntem: HESC ve 12Z insan endometriyal ve endometriyotik stromal hücre hatları, üreticinin talimatlarına göre üretildi. Hücresel empedansı belirlemek için xCELLigence (RTCA) cihazında empedans ölçümleri yapıldı. Hücrelere 10^{-6}-10^{-12} M arasındaki dozlarda N-asetil sistein uygulandı ve NAC için IC50 hesaplandı. Hücreler IC50 NAC ile muamele edildi ve

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was analyzed at 0, 24, and 48 hours. GRP78, ER tracker, and tubulin-specific immunofluorescent labelings were performed. The corrected total cell fluorescence (CTCF) was counted for each sample. The GraphPad Prism 8 program was used for statistical analysis. The Shapiro-Wilk test, Mann-Whitney U test, Kruskal-Wallis test, and posthoc Dunn tests were done.

Results: 12Z cell proliferation was decreased by 10^{-6}-10^{-12} M NAC, while HESC cell proliferation was unaffected. The IC50 for 12Z cells was determined as 3.87 x 10^{-9} M. The wound gap width of 12Z cells treated with NAC was substantially wider at 48 hours into the migration test than that of the control. N-acetyl cysteine treated 12Z cells exhibited increased ER-tracker and GRP78 fluorescent immunolabeling, but decreased tubulin labeling. N-acetyl cysteine did not significantly alter the immunofluorescence labeling for those proteins in HESC cells.

Conclusion: Endometriosis is a severe, ongoing condition that primarily affects females who are fertile and is linked to infertility. Therefore, we demonstrated that application of NAC in a time-dose and impedance-dependent manner helped decrease the proliferative and migratory capacity of endometriotic cells, lowering the expression of the tubulin protein, raising ER stress, and consequently raising the level of chaperone proteins. We can say that NAC may be a possible curative or supportive medication in the treatment of endometriosis.

Key Words: Endometriosis, n-acetyl cysteine, proliferation, migration, microtubule, endoplasmic reticulum, chaperone protein

yara iyileşmesi 0, 24 ve 48. saatlerde analiz edildi. GRP78, ER işaretleyici ve tubuline özgü immünofloresan işaretlemeler yapıldı. Her örnek için düzeltilmiş toplam hücre floresansı (CTCF) hesaplandı. İstatistiksel analiz için GraphPad Prism 8 programı kullanıldı. Shapiro-Wilk testi, Mann-Whitney U testi, Kruskal-Wallis testi ve posthoc Dunn testleri yapıldı.

Bulgular: 12Z hücrelerinin çoğalması 10^{-6}-10^{-12} M NAC ile azalırken HESC hücrelerinin çoğalması etkilenmedi. 12Z hücreleri için IC50, 3.87 x 10^{-9} M olarak belirlendi. NAC ile tedavi edilen 12Z hücrelerinin yara aralığı genişliği, migrasyon testinin 48. saatinde kontrolünkinden önemli ölçüde daha genişti. N-asetil sistein ile muamele edilmiş 12Z hücrelerinde, artan ER-işaretleyici ve GRP78 immünofloresan işaretleme gözlendi, ancak tübülin işaretlenmesi azaldı. N-asetil sistein, HESC hücrelerinde bu proteinler için immünofloresan işaretlenmesini önemli ölçüde değiştirmedi.

Sonuç: Endometriyozis, öncelikle üreme çağında olan kadınları etkileyen ve kısırlıkla bağlantılı ciddi, süreğen bir hastalıktır. Bu nedenle, NAC'nin zaman-doz ve empedansa bağlı şekilde uygulanmasının, endometriyotik hücrelerin çoğalma ve göç etme kapasitesini azaltmaya, tubulin proteininin ekspresyonunu düşürmeye, ER stresini arttırmaya ve sonuç olarak şaperon proteinlerinin seviyesini yükseltmeye yardımcı olduğunu gösterdik. N-asetil sisteinin endometriyozis tedavisinde olası bir iyileştirici veya destekleyici ilaç olabileceğini söyleyebiliriz.

Anahtar Kelimeler: Endometriyozis, n-asetilsistein, çoğalma, göç etme, mikrotübül, endoplazmik retikulum, şaperon protein

INTRODUCTION

Endometriosis is a chronic, incapacitating disease associated with infertility and pelvic pain. The disease is relatively prevalent among women of reproductive age, and 6–10% of women suffer from endometriosis (1). Endometriosis is the term for the condition in which endometrial glands and stromal tissue are visible outside of the uterine cavity (1, 2). Dysmenorrhea, dysparonia, heavy menstrual cycles, and nulliparity are the most common clinical outcomes (1). The etiopathogenic mechanisms involved in the

illness are still not fully known, despite the fact that the relationship between the disease and infertility is frequently studied in the literature (3). It is believed that the disease is influenced by a variety of genetic, environmental, and epidemiologic factors (1). There are a variety of hypotheses, including the ability of Müllerian duct-derived embryonic cells to survive in ectopic places and ectopic implants of menstrual shredded tissue and retrograde bleeding (4). Since there is a low diagnostic rate, many endometriosis lesions cannot be identified (5, 6). Apart from anti-inflammatory medications, analgesic medications, and laparoscopy, there hasn't been a curative therapy for the illness either. An increasing body of evidence suggests that atypical ovarian endometriosis may be the precursor to ovarian cancer (5). Because it lowers life quality and also results in infertility and cancer, endometriosis continues to be a major problem for women who are of reproductive age (7, 8).

N-acetylcysteine (NAC) is a novel pain reliever that has recently been studied by scientists. Because it replenishes glutathione (GSH), NAC has traditionally been used as an antidote for respiratory illnesses, acetaminophen overdoses, and, in rare cases, nephropathy (9, 10). It is known that oxidative stress is increased in endometriotic tissue compared to normal endometriotic tissue, and oxidative stress is also accepted as the progressive cause of the onset of endometriosis (10). N-acetylcysteine has been used in a limited number of studies for endometriosis treatment, both *in vivo* and *in vitro*. According to a study, using a combination drug containing NAC reduced inflammation and ectopic endometrial growth *in vivo* and *in vitro*, as well as relieving pain in endometriosis patients (11). N-acetylcysteine was also suggested as a promising treatment for endometriosis in an observational cohort trial by reducing the size and number of cysts (12) and improving oocyte quality in mice by increasing mitochondrial activity and decreasing oxidative stress (13).

Almost all eukaryotic cells' cytoskeleton, which is made up of microtubules (MTs), is formed by the dimerization of the tubulin proteins α- and ß-tubulin. The elongation, shortening, and halt phases of MT dynamics are important for intracellular trafficking, mitotic spindle formation, cytokinesis, cell membrane blebbing, cell migration, and phagocytosis (14).

Endoplasmic reticulum (ER) is an important organelle as being a large site of protein synthesis, and the abnormal accumulation of protein aggregates in the ER lumen is known as ER stress (15). GRP78 is an ER chaperone involved in regulating protein folding and ER transmembrane inducers. The expression of GRP78 and ER activity appear to be directly correlated (16, 17).

Since endometriosis is defined by the aberrant invasion of endometrium-like tissues and cells outside the uterus, ER stress stimulation might be used as a therapeutic strategy to reduce proliferation and migration in endometriotic cells. As NAC is well known for being an antioxidant and ROS scavenger (18), we sought to find out more about its impact on endometriotic cells in light of its anti-proliferative and anti-migratory properties and whether it could be related to ER stability and chaperone protein alterations. Here, we proposed that endometriotic cells exposed to the IC50 level of NAC would exhibit reduced proliferative potential as well as migratory behavior due to an in vitro decrease in tubulin expression and ER stability.

MATERIAL and METHOD

Cell culture

HESC (#T0533, ABM, Canada) and 12Z (#T0764, ABM, Canada) human endometrial and endometriotic stromal cell lines were cold-chain transferred into our laboratory and thawed immediately according to the manufacturer's instructions. HESC, an endometrial stromal cell line, was cultured in DMEM/F12 (#P04-41550, PAN-Biotech, USA) medium supplemented with 1% Pen-strep, 10% FBS, and 1% Pen-strep. The endometriotic cell line 12Z was grown in Prigrow IV (#TM004, ABM, Canada) medium with 10% FBS. The

cell lines were incubated at 37 °C in 5% CO_2 culture conditions.

Proliferation analysis by real-time impedance-based proliferation analysis (RTCA)

The cell culture medium was removed, and the cells were washed with PBS when the HESC and 12Z cell lines reached 70-80% confluence. The cells were then incubated for 10 minutes at 37 °C with a trypsin/EDTA solution (#T4049, Sigma-Aldrich, USA) before centrifugation at 1200 rpm. To seed 5000 cells per well, 200 microliters of pellet and medium mixture were seeded into 96 wells of gold electrode-covered "E plates." To determine the efficacy of NAC (N-Acetyl-L-Cysteine) (#A9165, Sigma-Aldrich, USA) on cell proliferation, impedance measurements were performed using the xCELLigence (RTCA, Roche Applied System, Switzerland) device. The "cell index" was created by continuously recording the impedance of the pressure of the cells to the base. N-Acetyl-L-cysteine was dissolved in medium (DMEMF12, Prigrow IV) of 1 mM concentration and applied to the cells after 24 hours at doses between 10^{-6}-10^{-12} M for 6 repetitions. For a total of 96 hours, cellular indices were recorded every 15 minutes. The IC50 for NAC was calculated through RTCA software.

Scratch and cell migration assay

Endometrial stromal cells and endometriotic cells were seeded as $1x10^5$ cells per well in 6-well plates. After the cells had adhered and reached 70-80% confluence, a cell-free area in each well was created by scraping a line with a sterile micropipette tip. The wells were washed with medium after scraping to remove non-adherent cells. The cells were then treated with IC50 NAC or only medium. The wound healing was analyzed by taking images from the wells at the same positions using a phase contrast microscope (IX-73, Olympus, Japan) at 0, 24, and 48 h. Each measurement was made with care to measure the same area, and the measurements were repeated at least three times.

The calculation of "percent wound confluence" was analyzed according to the formula:

A: initial scratch wound; B: scratch wound at the time.

% wound confluence = [(A-B)*100 %]/A

Immunofluorescence labeling

The cells were seeded $1x10^4$ per well in 8-well slides. When the cells reached a confluency of 70-80%, the medium was removed, and the cells were washed with PBS. The cells were then fixed in cold acetone for 10 minutes at 4 °C and air-dried for 30 minutes at room temperature. The cells were blocked with 5% skimmed milk and then incubated in a humidity chamber overnight at +4 °C with 1:100 dilutions of anti-human GRP78 (#ab212054, Abcam, UK), ER tracker (#E34251, Invitrogen, USA), and ß-tubulin (#T8328, Sigma-Aldrich, USA) primary antibodies. The cells were labeled with the secondary antibodies Alexa 488 and 594 (#ab150080, Abcam, UK; #ab150113, Abcam, UK). To stain nuclei, DAPI (#422801, Biolegend, USA) was applied, and then the slides were mounted with antifade mounting medium. The presence of specific immunolabeling was determined using a light microscope attached to a digital camera (Leica DM6B, Germany). Evaluation of the immunolabeling was performed via LASX software. At least three micrographs from non-overlapping areas of both positive and negative controls were measured for fluorescence, and the corrected total cell fluorescence (CTCF) was counted for each sample.

Statistical Analysis

The RTCA software was used for determining the IC50 of NAC. The migration and wound healing analyses were carried out using the % wound confluence formula. For immunofluorescence analysis, the corrected total cell fluorescence (CTCF) formula was used to calculate the measurements, and the GraphPad Prism 8 program was used for statistical analysis. Analytical methods were used to assess the variables' conformity to the normal distribution (the Shapiro-Wilk test). Mann-Whitney U tests were used

for pairwise comparisons, the Kruskal-Wallis test for multiple comparisons, and posthoc Dunn tests for posthoc Dunn tests. The total type-1 error level was set at 5% for statistical significance.

RESULTS

Under ideal conditions, human endometrial stromal cells (HESC) and human endometriotic cells (12Z) were cultured. Cells were examined under a phase contrast microscope every three days and noted as having the morphology of human stromal cells. They had long cytoplasmic extensions and euchromatic nuclei. For the experiments, care was taken to ensure that both cells' passages had the same passage number. It was discovered that 12Z cells grown in vitro had a greater proliferative capacity and reached confluence faster than HESC cells (Figure 1A, B).

In 96-well gold electrode-coated E plates, confluent cells were seeded. Both cells' "cell index" reached 1 after about 24 hours of seeding. We discovered that 12Z cells had a much higher cell index, which the RTCA software recorded (Figure 1C, E). At the 25th hour, N-acetyl cysteine was applied to the cells at appropriate rates, six times at doses determined to be 10^{-6}-10^{-12} M. To determine the effectiveness of NAC on cell proliferation, impedance measurements were taken using the xCELLigence (RTCA, Roche Applied System, Switzerland) device. Cellular indices were measured every 15 minutes for 96 hours. We discovered that although 10^{-6}-10^{-12} M NAC inhibited the proliferation of 12Z cells (Figure 1E), it did not affect the proliferation of HESC cells (Figure 1C). The cell index in NAC-treated 12 Z cells was found to be lower than in the untreated control group. The IC50 for 12Z cells was determined to be 3.87 x 10^{-9} M. After determining the IC50 for 12Z cells (Figure 1D). The cells were exposed to the IC50 dose of NAC for migration analysis. Phase contrast microscopy was used to analyze images taken from the wells at 0th, 24th, and 48th hours (Figure 2A). At the 0th hour, the wound width created by scraping in the wells did not differ significantly (p = 0.6480) between the 12Z control, 12Z NAC, and HESC control and HESC NAC groups. No significant difference was observed between the 12Z control, 12Z NAC, and HESC control and HESC NAC groups in the wound width measurements performed at the 24th hour of

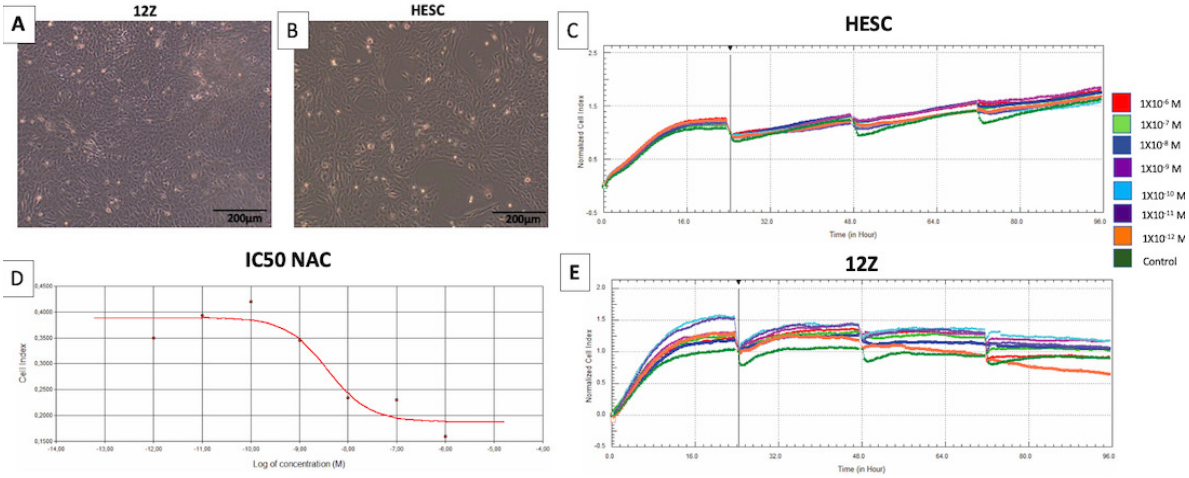

Figure 1. **(A)** Human endometrial stromal cells (HESC) and **(B)** Human endometriotic cells (12Z) displayed the morphology of human stromal cells with their extended cytoplasmic extensions and euchromatic nuclei. The 12Z cells were seen to have a higher proliferative capacity than HESC cells (10x magnification, scale bar 200µm). **(C, E)** The RTCA program observed that NAC had more anti-proliferative efficacy on 12Z cells than on HESC cells in 96-well gold electrode-coated E plates. **(D)** The IC50 for 12Z cells was 3.87 x 10^{-9} M.

the migration test (p = 0.0623). At the 48th hour of the migration test, measurements revealed that the wound gap width of 12 Z cells treated with NAC was significantly greater than that of the control group (p = 0.0057), but there was no significant difference in the wound gap width of HESC cells treated with NAC or medium (p= 0.9335) (Figure 2 B).

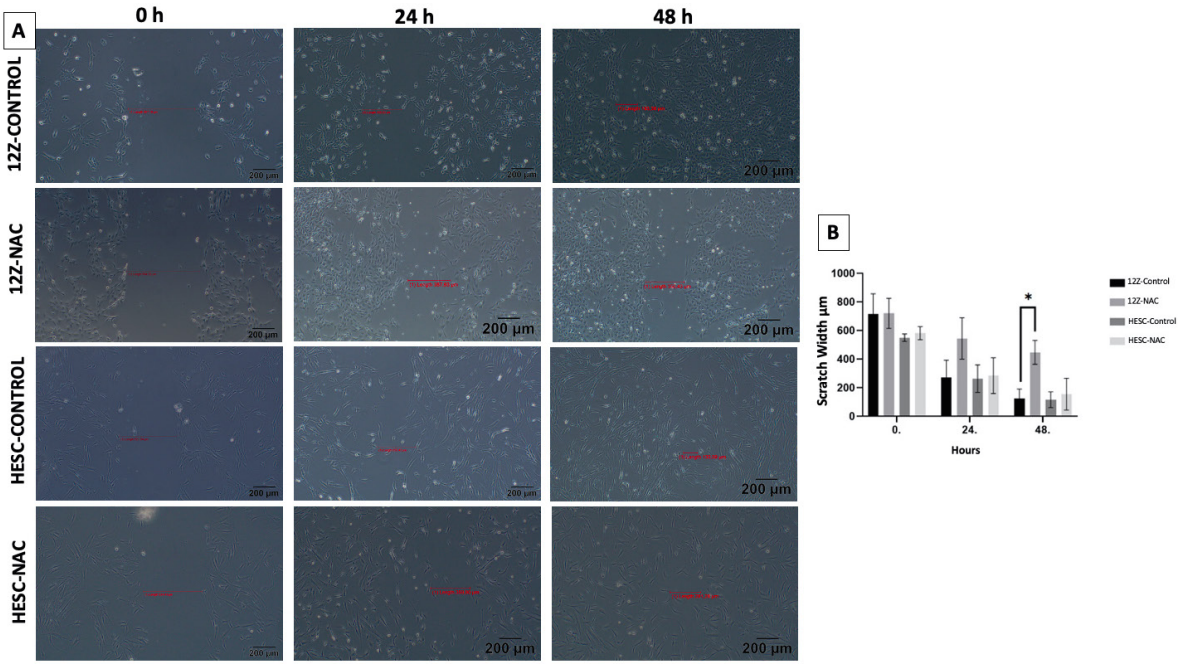

Figure 2. **(A)** For a 48-hour migration investigation, the IC50 dosage of NAC was administered to the HESC and 12Z cells (10x magnification, scale bar 200µm). **(B)** The wound gap width of 12 Z cells treated with NAC was significantly wider than that of the control group at the 48th hour of the migration test, respectively (*p < 0,05).

The cells were labeled with anti-human GRP78, ER tracker, and ß-tubulin primary antibodies (Figures 3, 4). The fluorescence labeling with ß-tubulin antibody was more intense around the perinuclear area of the cells (Figure 3, 4). The fluorescence intensity in at least three micrographs from non-overlapping areas of both positive and negative controls was measured, and the corrected total cell fluorescence (CTCF) was calculated for each sample. Fluorescent immunolabeling with ß-tubulin was found to be more intense in the 12Z control group. In addition, it was found that ß-tubulin labeling was statistically significantly higher than in NAC-treated 12Z cells (p= 0.0339) (Figure 3A, B, E). Endoplasmic reticulum membrane labeling with ER tracker was significantly higher in NAC-treated 12Z cells than in cells without NAC application (p= 0.0189) (Figure 3 A,B, and F). Immunofluorescence labeling of the ß-tubulin and ER tracker in HESC cells exposed to NAC or medium only revealed no significant differences (Figure 3C, D, G, and H).

When the 12Z control and NAC-treated groups were compared in GRP78 CTCF measurements, it was discovered that the immunofluorescent labeling in the NAC-treated group was significantly higher than in the medium treated group (p=0.0394) (Figure 4 A, B and E). Immunofluorescence labeling of GRP78 in HESC cells exposed to NAC or medium alone revealed no significant differences (Figure 4 C, D, and F).

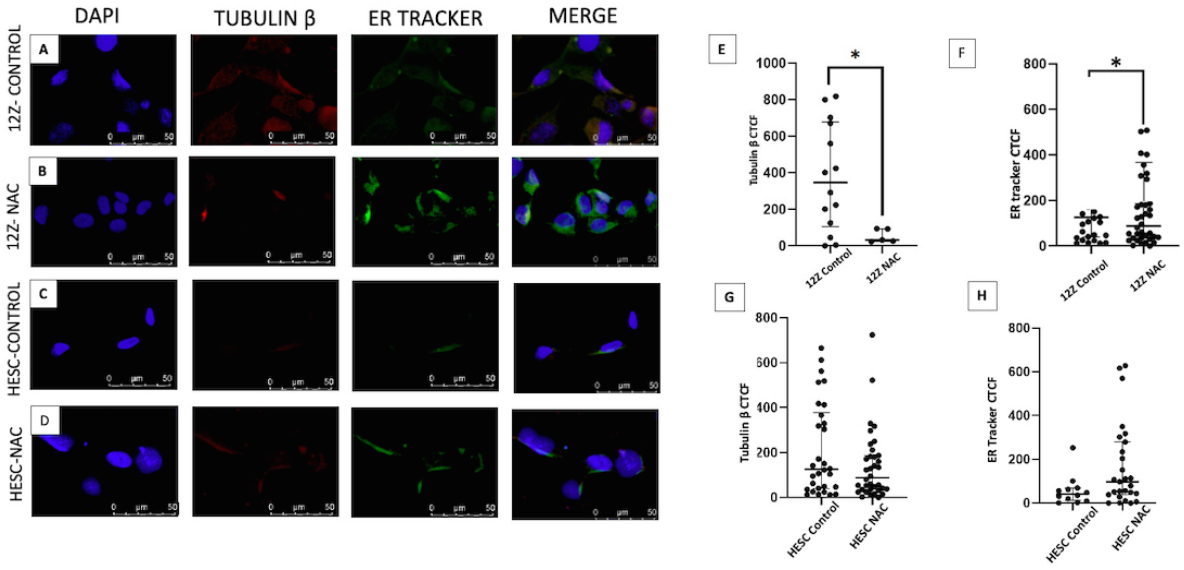

Figure 3. **(A)** Representative images of 12Z and HESC cells fluorescently labeled with DAPI (blue), ß-tubulin (red), and ER tracker (green) are shown in **(A, B, C, and D)** (FL, scale bar 50μM). **(E)** N-acetyl cysteine exposure was shown to decrease the ß-tubulin in 12Z cells compared to controls (*p < 0,05), and **(F)** Dramatically increased ER tracker labeling in 12Z cells compared to untreated 12Z cells (*p < 0,05). **(G, H)** Immunofluorescent labeling of ER Tracker and ß-tubulin in HESC cells did not identify any significant variations

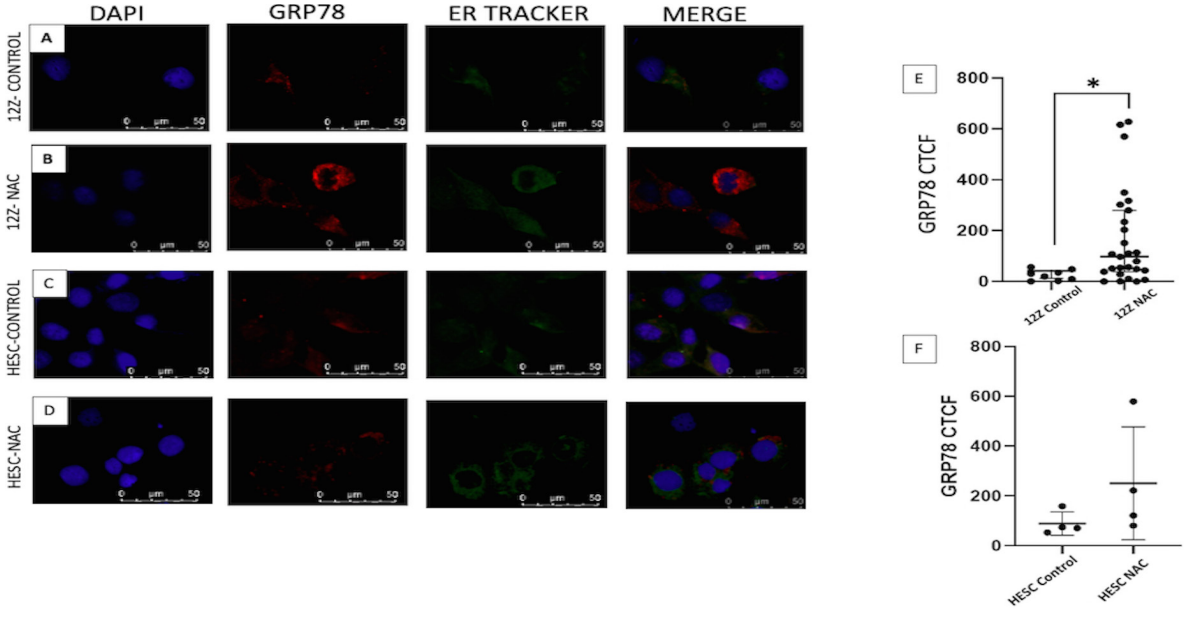

Figure 4. **(A)** Representative images of 12Z and HESC cells fluorescently labeled with DAPI (blue), GRP78 (red), and ER tracker (green) are shown in **(A, B, C, and D)** (FL, scale bar 50μM). **(E)** N-acetyl cysteine exposure was shown to increase the GRP78 labeling in 12Z cells compared to controls (*p < 0,05), and **(F)** There were no significant difference when GRP78 was immunofluorescently labeled in HESC cells that had been treated to NAC or just medium.

DISCUSSION

Endometriosis is a severe, chronic disorder that affects women of reproductive age and is associated with infertility and pelvic pain (1). The disease continues to be a major worry for women because of its detrimental consequences on life expectancy, elevated cancer risk, fertility, and reproductive health (1, 3, 4). In addition, there is no effective treatment method for this disease, other than painkillers, anti-inflammatory drugs and laparoscopy (5-8).

Recently, researchers analyzed the potential painkiller N-acetylcysteine (NAC) an antioxidant derivative of the amino acid L-cysteine. Although it has been used as an antidote for acetaminophen abuse, nephropathy, and rare cases of respiratory infections, NAC has only been explored in a few studies for endometriosis (9, 10). So far, the impact of NAC on endometriotic tissue and cells has been investigated, including how it affects oxidative stress, inflammation, pain, the size and weight of endometriotic lesions, and mitochondrial activity, both *in vivo* and *in vitro* (10-13). As a result, we reasoned that the *in vitro* application of IC50 NAC might have an anti-proliferative effect on the human endometriotic cells.

Under a phase contrast microscope, we discovered that human endometriotic cells (12Z) and human endometrial stromal cells (HESC) had similar morphologies and stromal cell features. Like fibroblast cells, the cells exhibited euchromatic nuclei and protracted cytoplasmic extensions. This fits in with the available literature (19). Moreover, we found that 12Z cells proliferated more actively and grew more rapidly than HESC cells. The greater proliferative activity of endometriotic cells *in vitro* was consistent with the results of other studies (20).

We gave cells a dosage screen of 10^{-6}-10^{-12} M, despite the fact that the majority of researchers supplied NAC in a non-dose-impedance dependent way. In this study, it was determined for the first time in research how NAC influenced endometrial and endometriotic cells throughout the course of time and dose. Due to the doses administered by the xcelligence system, the 12Z cells exposed to NAC tended to decrease their proliferative capacity, which is compatible with the previous studies (21, 22). Nevertheless, HESC cells did not change their ability to proliferate as a result of the NAC dosages used. Hence, we determined the IC50 for 12Z cells and discovered that it was 3.87 x 10^{-9} M. The IC50 dose of NAC was administered to 12Z cells and HESC cells in order to examine any potential anti-migratory effects. There was no difference in wound confluence between cell lines at the 0th hour due to NAC. Neither the 12Z control, 12Z NAC, nor the HESC control and HESC NAC groups' wound confluence at the 24th hour was significantly impacted by NAC. At the 48th hour, the wound width measurements between the 12Z control group and the 12Z NAC-applied group were significantly different. At 48 hours, we discovered that the wound confluence of the 12Z cells in the control group was significantly higher than the wound confluence of the 12Z cells in the NAC-applied group. This was in line with the findings of Woo et al.'s study, which revealed that 5 mM NAC reduced the migration capacity of 12Z cells at 24 hours (23). In addition, Su et al. reported that 2 mM NAC inhibited the migration of the endometrial cancer cell line Ishikawa's increased migratory activity due to ROS elevation (24).

We presumed that the potential anti-proliferative and anti-migratory influence of in vitro NAC application on endometriotic cells could also result in a reduction in tubulin expression due to the significance of "microtubule dynamics" in cell movement, migration, and the formation of mitotic spindles. In a non-small cell lung cancer cell line, NAC was demonstrated to decrease α- and ß-tubulin expression as well as to restrict migration raitos (25); however, the main goal of our investigation on endometriotic cells was to use IC50 and to inhibit migration raitos amid decreased ß-tubulin expression. In this study, we showed that ß-tubulin fluorescent labeling was present throughout the cytoplasm and around the perinuclear region of endometrial and endometriotic cells. In comparison to the 12Z NAC-applied group, the beta tubulin fluorescent immunolabeling was shown to be stronger in the 12Z control group. As a result, we can conclude that IC50 NAC; decreased ß-tubulin protein expression and migration in endometriotic cells over a 48-hour period.

Since NAC has been shown to lessen ER stress in the literature (26), we wanted to find out if endometriotic cells exposed to IC50 NAC exhibited ER stability or instability. Also, we looked into the GRP78 labeling by immunofluorescence in order to see if the process had altered the chaperone protein in the endometrial and endometriotic cells, in addition to ER tracker labeling (14, 16, 17). There were no significant differences between HESC cells treated with NAC or media alone when the ER tracker was immunofluorescence labeled. In 12Z cells, without NAC compared to cells with NAC, ER tracker labeling of the endoplasmic reticulum was considerably reduced. The immunofluorescent labeling of GRP78 in the NAC-treated group was substantially higher than in the medium-treated group in 12Z cells. No significant differences were seen when HESC cells treated to NAC or only media were labeled with immunofluorescence for GRP78.

Contrary to previous studies (26, 27), NAC induced ER stress on endometriotic cells in our study. In previous studies, it was indicated that NAC would reduce ER stress and scavenge ROS intracellularly (27). However, we found that the IC50 for NAC should be used at a nanomolar concentration in the current study. Given the majority of earlier research relied on milimolar to micromolar doses of NAC, it's possible that just this dosage increased the endometriotic cells' ER stress. As a result, utilizing NAC in a time-dose and impedance-dependent manner helps lower the cell's proliferative and migratory capacity, lowers the expression of the tubulin protein, raises ER stress, and consequently raises the level of chaperone proteins.

From this point forward, we can state that NAC application in a time- and dose-dependent manner can be helpful for treating endometriosis, and if we can complete further in vivo and in vitro functional quantitative studies, NAC could be a potential curative or supportive agent.

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ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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