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Kolorektal Kanser Hücrelerinde Mentha Longifolia ve Dosetaksel Kombine Tedavisinin DNA Tamir Mekanizması Üzerindeki Etkisi

Mentha Longifolia And Docetaxel Combined In Colorectal Cancer Cells The Effect of Treatment on DNA Repair Mechanism

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ÖZET

Amaç: Kolorektal kanser, dünya genelinde yüksek morbidite ve mortalite oranlarına sahip yaygın bir malignitedir. Mevcut kemoterapötik ajanlara karşı gelişen direnç, alternatif veya destekleyici tedavi stratejilerine olan ihtiyacı artırmaktadır. Bu çalışmada, doğal bir bitki olan *Mentha Longifolia* ekstresi ile kemoterapötik bir ajan olan Dosetaksel'in kombinasyonunun, kolorektal kanser hücrelerinde DNA tamir mekanizmaları üzerindeki etkisi araştırılmıştır.

Yöntem: Çalışmada özellikle DNA hasar onarımında kritik rol oynayan *ERCC1* (Eksizyon Onarım Çapraz Tamamlayıcı Grup 1) ve hücresel stres yanıtında önemli görevleri bulunan *ATR* (Ataksi Teleanjiektazi ve Rad3 ile ilgili) genlerinin ekspresyon düzeyleri değerlendirilmiştir.

Bulgular: Kolorektal kanser hücreleri üzerinde uygulanan kombine tedavi, tek başına uygulanan bileşenlere kıyasla DNA tamir genlerinin ekspresyonunda anlamlı değişikliklere neden olmuştur.

Sonuç: Bu sonuçlar, *Mentha Longifolia'nın* Dosetaksel ile birlikte uygulanmasının DNA tamir yollarını hedef alarak tedavi etkinliğini artırabileceğini ve böylece kemoterapiye direnç gelişimini azaltabileceğini düşündürmektedir. Bulgular, kolorektal kanser tedavisinde bitkisel kökenli ajanların potansiyel destekleyici rolünü ortaya koymakta ve gelecekteki klinik çalışmalar için temel oluşturmaktadır.

Anahtar kelimeler: DNA tamir mekanizmaları, kolon kanseri, *Mentha Longifolia*, kombine tedavi.

ABSTRACT

Objective: Colorectal cancer is a common malignancy with high morbidity and mortality rates worldwide. The emerging resistance to current chemotherapeutic agents increases the need for alternative or supportive treatment strategies. In this study, the effect of the combination of *Mentha Longifolia* extract, a natural plant, and Docetaxel, a chemotherapeutic agent, on DNA repair mechanisms in colorectal cancer cells was investigated.

Methods: In the study, the expression levels of *ERCC1* (Excision Repair Cross-Complementation Group 1), which plays a critical role in DNA damage repair, and *ATR* (Ataxia Telangiectasia and Rad3-related) genes, which have important roles in cellular stress response, were evaluated.

Results: The combined treatment of colorectal cancer cells caused significant changes in the expression of DNA repair genes compared to the components administered alone.

Conclusion: These results suggest that the co-administration of *Mentha Longifolia* with Docetaxel may improve treatment efficacy by targeting DNA repair pathways and thus reduce the development of resistance to chemotherapy. The findings reveal the potential supportive role of plant-based agents in the treatment of colorectal cancer and provide a basis for future clinical trials.

Keywords: DNA repair mechanisms, colon cancer, *Mentha Longifolia*, combination therapy..

INTRODUCTION

Cancer is a disease characterized by uncontrolled proliferation of cells due to environmental and genetic factors.[1] Although the overall survival time and early detection time of the disease have shortened, cancer-related mortality is the second leading cause of death worldwide.[2] Approximately 19.98 million people worldwide have been diagnosed with cancer and 9.74 million people have been reported to have died due to cancer. Among cancer types, lung cancer is the most common cancer with 2.48 million new cases (12.4%), followed by breast cancer (11.5%) and colorectal cancer (9.6%).[3,4] Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide and the second leading cause of cancer-related deaths.[5] The 5- and 10-year survival rates of CRC are reported to be 65% and 58%, respectively.[6] The global incidence of colorectal cancer is predicted to increase significantly in the coming years.[7] The vast majority of colorectal cancer cases are sporadic; however, a small proportion are associated with genetic disorders. However, lifestyle factors and the aging process play an important role in the development of CRC.[8,9,10]

Despite the emergence of advanced treatment modalities such as immunotherapy and gene therapy, surgery, radiotherapy, chemotherapy and hormonal therapy are still the most commonly used treatment modalities due to their cost-effectiveness and widespread use.[11] Each of these modalities has its own specific disadvantages and side effects.[12,13] In particular, local tumor recurrence and development of distant metastasis are common complications encountered during the treatment process in many CRC patients and pose significant challenges in disease management.[14,15] Therefore, researchers continue their efforts to develop more effective and safer treatment strategies to overcome these challenges.[16]

In recent years, phytotherapy, in which extracts obtained from herbal sources are used, has come to the forefront among alternative treatment approaches.[17,18] *Mentha* genus plants belonging to the Lamiaceae family are evaluated as phytotherapeutic agents. Anti-obesity, antimicrobial, anti-inflammatory, anti-diabetic and cardioprotective effects of these species have been demonstrated in scientific studies.[19] The main components of essential oils obtained from *Mentha* species include alcohols, ketones, esters, ethers and oxides.[20,21] In addition, *Mentha* genus and many similar medicinal plants have antioxidant capacity and reduce the effects of oxidative stress with molecules such as phenolic compounds, ascorbic acid and carotenoids. Reported that *Mentha Longifolia* had the highest antioxidant activity among nine different *Mentha* species in their study.[22]

Figure 1. *Mentha Longifolia L.*

Plants have historically been used to treat many diseases, including cancer, and more than 60% of currently used anti-cancer drugs are derived from natural sources. These natural anti-cancer agents include vinca alkaloids, taxanes and analogs, podophyllotoxin. and derivatives,

camptothecin (CPT) and derivatives, anthracyclines and others.[23,24] Docetaxel, one of the chemotherapeutic agents used in colorectal cancer treatment, is an anti-mitotic taxane class drug. Taxanes inhibit microtubule dynamics by binding to β-tubulin and trigger apoptosis by regulating cell division.[25,26]

Loss of function of DNA repair mechanisms plays an important role in cancer development.[27,28] Colorectal cancer is characterized by genetic defects in DNA repair genes at both germline and somatic levels.[29,30] There are six major DNA repair pathways in human somatic and germ cells: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR), non-homologous end joining (NHEJ) and translational DNA synthesis (TLS).[31] Among these mechanisms, Excision Repair Cross Complement 1 (ERCC1), an important enzyme that repairs platinum-DNA complexes by showing 5' endonuclease activity in the nucleotide excision repair (NER) pathway, is a 15.3 kb gene located on 19q13.2-q13.3 encoding a 33 kDa protein of 297 amino acids.[31,32,33] ERCC1 has been reported to play a critical role in treatment resistance to platinum-based chemotherapy and is therefore considered as a potential prognostic marker in colorectal cancer.[32,33]

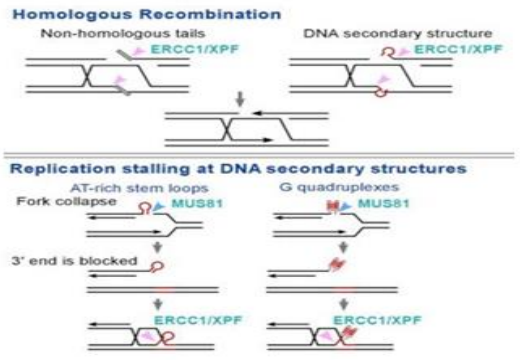

Figure 2. Repair mechanism of ERCC1.[26]

Another important protein involved in DNA repair is Ataxia Teleangiectasia and Rad3-Related 3 (ATR) kinase. ATR is activated in the presence of single-stranded DNA coated by replication protein A (RPA) and ensures stabilization of replication forks and cell cycle arrest.[34]

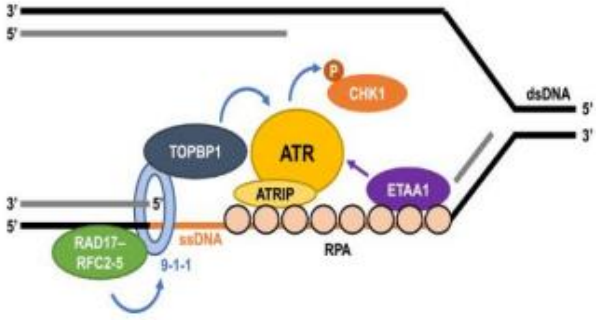

Figure 3. ATR repair mechanism.[39]

Another important protein involved in DNA repair is *Ataxia Teleangiectasia and Rad3-Related 3 (ATR)* kinase. ATR is activated in the presence of single-stranded DNA coated by replication protein A (RPA) and ensures stabilization of replication forks and cell cycle arrest.[35]

MATERIAL and METHODS

Plant material

Mentha Longifolia L. Used in the study was collected from Tepeköy and Ütük villages of Zara district of Sivas province in two different periods in May and September 2023.

Preparation of the extracts

The essential oils of the plants were extracted separately as flowerless whole stem and flower+stem. Essential oils were obtained by hydrodistillation of the specified parts of the plants in the Clevenger apparatus for 3 hours. The condenser part of the Clevenger apparatus was connected to the microhiller and the cooling water was kept at 4°C. The yield of the oil obtained was determined as v/W (mL oil/g plant). The isolated essential oil was purified from

the water it contained on Na_2SO_4 and transferred to amber bottles to be stored until the day of analysis and stored at -20°C.[36]

Cytotoxicity activites

The cytotoxic activity of *Mentha Longifolia* on HT-29 cell line was analyzed using 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) method. RPMI medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and 1% L-glutamine was used for cell lines. Cell lines were incubated at 37 °C and 5% CO_2. Cells ($1x10^5$/well) were seeded in 96-well plates and after 24 hours of growth, the indicated compounds were applied to the cells at concentrations of 250, 100, 50, 25, 10.5, 10.5, and 0.5 µM. Cells were exposed to *Mentha Longifolia* for 24, 48 and 72 hours of incubation. At the end of these periods, MTT assay was performed. Therefore, 10 µL of MTT prepared in phosphate buffered saline at a concentration of 5 mg/ml was added to each well and incubated for 3 hours at 37 °C in an environment containing 5% CO_2. After aspirating the medium containing MTT, dimethyl sulfoxide (DMSO) was used to dissolve formazan crystals. 100 µL of DMSO was added and incubated for 15 minutes at room temperature and absorbance values were read with a microplate reader UV spectrophotometer at a wavelength of 540 nm. These measurements were performed in 3 replicates for each sample and repeated at least three different passage numbers. IC_{50} values were calculated using GraphPad Prism and graphs were generated.

Cell culture

Cell lines including HT-29 RPMI medium, containing 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (10 mg/L). Cells were grown in at 37°C, 5% CO_2 and 95 % air in a humidified incubator. For each cell line, 70-80% confluent cell culture flask was trypsinized and cells were seeded in 6 well plates.

Statistical analysis

All experiments were carried out in triplicates and results are expressed as means ± standard error mean. Data were analyzed using one-way analysis of variance and differences were considered significant at $p < 0.05$. The IC_{50} were determined by statistical software, GraphPad Prism8 (GraphPad Software, San Diego, CA, USA).

DISCUSSION

The cytotoxic effects of combination treatment of *Mentha Longifolia* extract and Docetaxel on colorectal cancer cells and the effects on *ATR* (ataxia telangiectasia and Rad3-related) and *ERCC1* (excision repair cross-complementation group 1) gene expression levels, which play a critical role in DNA damage response, were investigated. The findings revealed that the combination treatment both significantly reduced cell viability and suppressed the expression of these two key genes involved in DNA repair. The decrease in cell viability observed in cytotoxic assays suggests that the phenolic compounds and flavonoids contained in *Mentha Longifolia* show a synergistic anticancer effect with the microtubule stabilizing effect of docetaxel.[37] Docetaxel arrests the cell cycle in the G2/M phase and induces apoptosis through stabilization of mitotic spindle strands.[38] It has been previously reported that herbal compounds may contribute to this effect by suppressing cell proliferation and increasing oxidative stress and DNA damage.[39] *ATR*, which is involved in the

DNA damage response, is particularly known for its activation in replication stress and single-stranded DNA regions.

CONCLUSION

In this study, the observed decrease in *ATR* gene expression suggests that combination therapy weakens the response of cells to DNA damage. This may contribute to cells being directed to apoptosis due to the inability to repair DNA damage.[40] Similarly, the *ERCC1* gene is a key component of the nucleotide insertion repair (NER) pathway and plays an important role in resistance to platinum-based and DNA cross-linking drugs. It is thought that decreased ERCC1 expression may increase sensitivity to docetaxel treatment. *Our findings suggest that this suppression in the expression of DNA repair genes is associated with the weakening of intracellular defense mechanisms against treatment and thus may increase the efficacy of combination therapy. Targeting repair genes such as ATR and ERCC1 may be an important strategy, especially considering the common problem of chemotherapy resistance in colorectal cancers.However, the limitation of this study is that it was performed in vitro. Confirmatory analyses at the protein level (Western blot, immunohistochemistry) and testing the efficacy in in vivo models will reinforce the clinical validity of the results.*

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