Orijinal Araştırma / Original Investigation
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Cytotoxic Effect of Teranecrone and Doxorubicin on Human Colorectal Adenocarcinoma (CaCo-2) Cell Line

İnsan Kolorektal Adenokarsinom (CaCo-2) Hücre Hattında Teranekron ve Doksorubisinin Sitotoksik Etkisi

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ÖZET

AMAÇ: Bu çalışmada veteriner hekimlikte sıklıkla kullanılan teranekronun insan kolorektal adenokarsinomu üzerinde antineoplastik etkinliğinin değerlendirilmesi amaçlandı.

GEREÇ VE YÖNTEM: Çalışmada etkinliğini değerlendirmek için teranekron (TRN) ve pozitif kontrol olarak da antineoplastik ilaç olan doksorubisin kullanıldı. İnsan kolorektal hücre hattı CaCo-2 üzerinde teranekronun sitotoksik etkinliği araştırıldı. Canlılık oranları belirlenmesinde MTT proliferasyonu testi uygulandı. İstatistiksel analizler için ise ANOVA Tukey testi kullanıldı ve anlamlılık düzeyi P<0,01 olarak kabul edildi.

BULGULAR: Canlılık testleri yaptığımızda teranekronun tek olarak uygulandığı gruplarda teranekronun doza bağlı olarak toksisitesinin arttığı görüldü. 10 µM konsantrasyonda CaCo-2 canlılığı %94 iken 100 µM'lık konsantrasyonda canlılık oranı % 79 a düştü. Sadece pozitif kontrol olarak uyguladığımız Dox 40 µM konsantrasyonunda canlılık %78,72 Dox 40 µM + TRN 100 µM uygulandığı grupta ise canlılık oranı %73 olarak belirlendi. Dox+TRN'nin birlikte uygulandığı gruplarda TRN 50 µM dozundan itibaren toksik etki oranı konsantrasyona bağlı olarak artmaya başladı.

SONUÇ: teranekronun CaCo-2 hücre hattı üzerinde yapılan canlılık testi sonucunda doza bağlı olarak TRN'nin sitotoksik etkinliğinin arttığı belirlendi. teranekron etkinliği antineoplastik ilaç olarak kullanılan doksorubisin ile karşılaştırıldığında ise 75 µM TRN'nin 40 µM Dox ile aynı etki ortay çıkarttığı belirlendi. Yan etki profilleri düşünülecek olursa daha az yan etki gösteren TRN insan kolorektal kanser tedavisinde alternatif bir tedavi olma potansiyeli olduğu görülmektedir. TRN'nin antikanser tedavisi kullanılmasının tavsiye edilmesi için daha detaylı çalışmalara ihtiyaç bulunmaktadır.

Anahtar Kelimeler: CaCo-2, doksorubisin, insan kolorektal adenokarsinom, teranekron,

ABSTRACT

OBJECTIVE: In this study, it was aimed to evaluate the antineoplastic efficacy of teranecron, which is frequently used in veterinary medicine, on human colorectal adenocarcinoma.

MATERIALS AND METHODS: Teranecron (TRN) was used to evaluate its effectiveness in the study, and the antineoplastic drug doxorubicin was used as a positive control. The cytotoxic activity of teranecron on the human colorectal cell line CaCo-2 was investigated. MTT proliferation test was used to determine the viability rates. ANOVA Tukey test was used for statistical analysis and the level of significance was accepted as P<0.01.

RESULTS: When we performed viability tests, it was observed that the toxicity of teranecron increased in a dose-dependent manner in the groups in which teranecron was administered alone. While the viability of CaCo-2 at 10 µM concentration was 94%, the viability rate at 100 µM concentration decreased to 79%. The viability was determined as 78.72% in the Dox 40 µM concentration, which we applied only as a positive control, and 73% in the group in which Dox 40 µM + TRN 100 µM was applied. In the groups where Dox+TRN was administered together, the toxic effect ratio started to increase depending on the concentration, starting from the TRN 50 µM dose.

CONCLUSION: As a result of the viability test of teranecron on the CaCo-2 cell line, it was determined that the cytotoxic activity of TRN increased in a dose-dependent manner. When teranechron efficacy was compared with doxorubicin used as an antineoplastic drug, it was determined that 75 µM TRN had the same effect as 40 µM Dox. Considering the side-effect profiles, TRN with fewer side effects seems to have the potential to be an alternative treatment for human colorectal cancer. More detailed studies are needed to recommend the use of TRN as an anticancer therapy.

Keywords: CaCo-2, doxorubicin, human colorectal adenocarcinoma, teranecrone,

INTRODUCTION

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Homeopathic treatment is among the alternative medicine applications. The main principles of this treatment are 'Similar things can be treated with similar ones' and 'When a substance is given to healthy people, whichever disease it causes similar symptoms in those people, sick people who actually have that disease can be treated with this active substance' (1) . Due to its low side-effect profile, homeopathy has been increasing in popularity since the end of the 18th century, when it was first defined, and studies on homeopathic treatment are increasing in every field (2). There are many studies investigating the effects of homeopathic treatment in human colorectal adenocarcinoma (3-5).

Teranechron® (TRN) is a homepoitic substance used in veterinary medicine. It is an alcohol extract of the spider venom of the Tarantula cubensis species (6). TRN has a supportive effect in the treatment of inflammation and necrotic processes (7). It also has anti-edema effects (8). It has been reported that breast trauma and eczema are also successfully treated with TRN (8).

Doxorubicin (Dox), which is an anthracycline class antineoplastic, is an antibiotic with strong cytotoxicity and a topoisomerase-II enzyme inhibitor that is frequently prescribed in cancer treatment (9). Dox is widely used to treat children and adults in cancer types such as breast and ovarian cancer, leukemia, and lymphoma (10).

Human colorectal cancer (also called rectal cancer or colon cancer) is one of the most common cancer deaths worldwide, regardless of gender (11). Although clinical trials conducted around the world have tried many new strategic treatments to overcome the limitations of conventional cancer therapy, this type of cancer is difficult to treat and many cases are fatal (12). The human colorectal cancer cell line (CaCo-2) is frequently used in many studies on this type of cancer.

In our study, the CaCo-2 cell line was used. The effects of TRN and Dox on this cell line were evaluated. MTT proliferation and viability test were used to evaluate the results.

MATERIAL & METHODS

Reagents

The materials used in the study are respectively; The alcoholic extract of Tarantula cubensis was obtained from Theranekron Richter Pharma (Wels, Austria), doxorubicin hydrochloride from Koçak Farma (Istanbul, Turkey), and the human colorectal adenocarcinoma cell line was obtained from ATCC (Manassas, VA, USA).

Cell culture model

CaCo-2 cells grown in 25 mL flasks were seeded into 96-well flasks with 5000 cells per well. At least 8 wells were used for each dose and substance. The CaCo-2 cell line planted in 96 flasks was incubated in an incubator at 37°C containing 5% CO2 (13). The experiment was started when cells filled 80% of these flask wells. Concentrations used in the experiment were added at 10, 25, 50, 75, 100 µM doses for TRN. Dox 40 µM (This dose has been adjusted according to the approximate IC50 dose specified in the literature. (14)) was used as positive control and only medium was used in the wells used as negative control. Again, in the TRN+Dox combination, the CaCo-2 cell line was added to the wells planted at the dose of TRN 10, 25, 50, 75 and 100 µM and Dox 40 µM. Each of the given doses was homogenized separately in 1000 µL medium and applied to the cells as 100 µL per well. Only 100 µL of medium was added to the negative control group.

MTT Test

MTT proliferation assay was used to determine cell viability. Based on this MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] test, it reacts by forming purple colored formazan crystals in the medium depending on mitochondrial reductase enzyme activity. After 24 hours of drug application, the medium in the 96-well plate is removed from the wells. After the medium was removed, 10 µL of the prepared MTT solution was added to each well and the total volume was completed to 100 µL with the medium. After this treatment, it was incubated for 4 hours for the formation of formazene crystals. After 4 hours of incubation, the solution and medium mixture was removed from the wells. To dissolve the formazan crystals, DMSO was added to the wells and when the formazan crystals were completely dissolved, reading was made with a Microplate Reader at 450 nm wavelength (15).

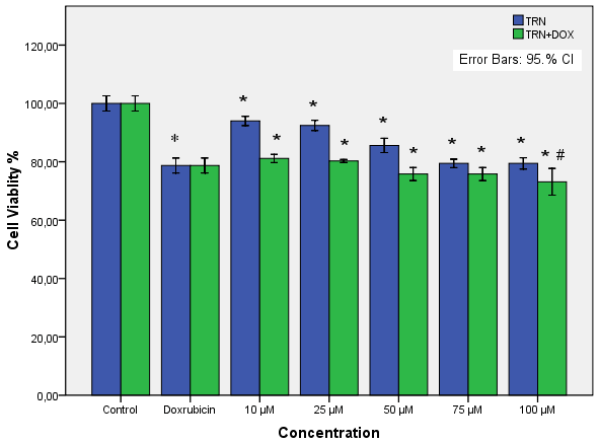
Statistical analysis

SPSS version 17 was used for statistical analysis. One-way analysis of variance (ANOVA) was used in the analysis of the data and Tukey test was performed. The results obtained were proportional to the negative control group and determined as a percentage. Percentages were given as mean ± standard deviation. $P<0.001$ was considered significant in the analyses .

RESULTS

When we evaluated the results of MTT analysis using the human colorectal adenocarcinoma (CaCo-2) cell line, TRN increased cell death depending on the concentration. While the viability was 94±1.5% at 10 µM, the viability decreased to 79.5±1.5% at the 100 µM concentration. This result showed that TRN decreased cell viability depending on the concentration. Another application was the application of the anticancer drug doxorubicin (Dox) together with TRN. Cell viability was measured as 78.72±2.77% with Dox applied as a positive control. Viability rates were also measured with the concentrations applied together with TRN at 5 different concentrations of 10-100 µM. There was no positive effect on cell viability of Dox applied alone and Dox applied together with TRN at concentrations of 10-75 µM. There was only difference in administration of 100 µM TRN+Dox. While there was 79.45±1.54% viability in the 100 µM application of TRN, the viability decreased to 73.14±3.69% in the TRN+Dox 100 µM application, and the viability was 78.72±2% in the Dox application alone. was 77 (Figure. 1). While only Dox (40 µM) and TRN 100 µM alone application decreased viability at a similar rate, this rate increased even more in TRN+Dox 100 µM application. However, it is not possible for us to make any comments about whether there is any summative effect between the two drugs. As a result, both substances have a toxic effect on the COCA-2 cell line. This effect is statistically significant (P<0.001). However, the efficiency is not very high, approximately 20% cell death has been observed.

Figure 1. *Results of CaCo-2 Cell line MTT analysis.*



*The doses applied only Teranecrone and the concentrations where TRN was applied together with Trenakron and Doxorubicin were indicated with TRN+Dox. The concentration of doxorubicin was 40 µM and its concentration was kept constant in all groups administered Dox. * P<0.01 compared to control, # P<0.01 according to doxorubicin was considered significant.*

DISCUSSION

In our study, the effects of Teranectone and doxorubicin on CaCo-2 human colorectal adenocarcinoma cell line were evaluated by viability test. In this study, the cytotoxic effect of teranecrone mono and docrubicin on the CaCo-2 cell line was investigated for the first time.

Teranecron is used in different types of cancer in veterinary medicine and has been investigated in many in vivo and in vitro studies. Animal studies in benign tumors have shown that teranecron may be effective in proliferative diseases. It has been reported that it is effective in achieving complete remission in skin papillomatosis in teranechronous cattle (16), and it is more effective in nipple papillomatosis than levamisole used in the treatment of papillomatosis (17). In another study, in canine oral papillomatosis in which surgical and/or systemic chemotherapeutics were used in the treatment, TRN achieved complete remission in all cases after 5 weeks of treatment (18). It has also been reported that TRN is effective in canine breast cancer (19, 20).

There are also in vitro studies using different cancer cell lines. In particular, many studies have been conducted to investigate cell death mechanisms. It has been reported that 6 hours of TRN administration increases apoptosis in MCF-7 cells (21). In a study using human breast tissue cancer cell line (MCF-7), human head and neck cancer cell line (HN-5) and human embryonic kidney cell line (HEK293) as healthy control, the cytotoxic effects of TRN were evaluated and dose-dependent cell line was determined. It has been reported to reduce proliferation (22). In this study, it was shown that the cytotoxic effect of TRN on cancer cell lines (especially MCF-7) is greater than that of the normal cell line, HEK293. In addition, DNA fragmentation was detected in these cancer cell lines (HN-5, MCF-7), but DNA fragmentation was not observed in the negative control group (HEK293). In order to explain the mechanism of apoptosis, caspase-3 level and activity were evaluated. As a result, it has been reported that the level of caspase-3 increases in all cells treated with TRN and that caspase-3 activity increases more in cancer cells compared to normal cells and induces apoptosis (22). It has been reported that TRN endogenous, extrinsic and endoplasmic reticulum-mediated signaling pathways induce apoptosis by oxidative stress and exert cytotoxic effects against MCF-7, A549 and Saos-2 cell lines (6).

In our study, it was determined that the cytotoxic effect of TRN increased depending on the dose. However, this cytotoxic effect was not seen more than the toxic effect of Dox. The 40 µM concentration of Dox and the 75 and 100 µM concentrations of TRN induced cell death at similar rates. This ratio was found to be approximately 79% for Dox, TRN 75 and 100 µM concentrations. In the doses where TRN+Dox was applied together, the viability percentages were found to be 75%, 75% and 73%, respectively, at the doses where TRN was applied as 50 µM, 75 µM and 100 µM. It was observed that TRN increased cell death in a dose-dependent manner, especially after a concentration of 50 µM, at doses co-administered with Dox (Figure 1).

CONCLUSION

As a result, TRN was applied to CaCo-2 cell lines in 5 different concentrations between 10-100 µM and its cytotoxic effect was evaluated by MTT proliferation test. It was observed that the toxic effect of TRN increased depending on the dose. This cytotoxic effect increased at the doses where TRN was administered together with Dox. However, the available data were not sufficient to make a sound assessment of whether there was a synergistic effect at the doses where the two substances were applied together. There are limitations in our study. This study was evaluated with a single analysis parameter. More detailed and detailed molecular mechanisms need to be evaluated. The lack of these detailed tests is a limitation of our study. It may be useful to investigate the synergistic effect in future studies. In addition, detailed research on TRN may be beneficial, especially in cancer treatment. TRN alone has potential as an alternative treatment to Dox for human colorectal adenocarcinoma due to the side-effect profiles seen in antineoplastic drugs.

Etik: Bu çalışmanın etik kurulu alınmıştır.

Ethics committee approval had been taken.

Yazar katkı durumu; Çalışmanın konsepti; NT, MSE, dizaynı; NT, MSE, Literatür taraması; NT, MSE, verilerin toplanması ve işlenmesi; NT, MSE, istatistik; NT, MSE, yazım aşaması; NT, MSE,

Author contribution status; The concept of the study; NT, MSE, design; NT, MSE, literature review; NT, MSE, collecting and processing data; NT, MSE, statistics; NT, MSE, writing phase; NT, MSE,

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