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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

KANSER TEDAVİSİNDE KULLANILAN ANTİ-MİKROTÜBÜL İLAÇLARDAN VİNCA ALKALOİDLER VE PAKLİTAKSEL'İN DNA BAĞLANMA AKTİVİTELERİ

DNA BINDING ACTIVITIES OF THE VINCA ALKALOIDS AND PACLITAXEL AS ANTI-MICROTUBULE DRUGS USED IN CANCER THERAPY

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ÖZET

AMAÇ: Mikrotübüller, hücre içi hücre iskeleti yapısının önemli bir parçasıdır ve hücre bölünmesi dahil birçok hücresel işlev için kritik olan benzersiz polimerizasyon dinamiklerine sahiptirler. Mikrotübüllere müdahale eden anti-mikrotübül ilaçlar, çeşitli kanserlerin tedavisi için çok önemli kemoterapötik ajanlardır. Mitozu bloke eden bu ilaçlar, mikrotübüllerin dinamiğini baskılayan, hücreleri yavaşlatan, apoptozu indükleyen ve ardından tümör hücrelerini öldüren ortak bir mekanizma ile çalışmaktadır. Vinka alkaloidleri (vinblastin, vincristin ve vinorelbin) ve Taksanlar (paklitaksel), mikrotübüllerin işlev bozukluğuna neden olan ve kanser hücresi proliferasyonunu inhibe eden iki farklı anti-mikrotübül ilaç sınıfıdır. Vinka alkaloidlerinin ve taksanların ana aktivitesi, tübülin proteinleri ile bağlanma etkileşimlerinden kaynaklanmaktadır. Ancak, bu anti-mikrotübül ilaçların DNA etkileşimleri ile ilgili çalışmalar yeterli değildir. Bu çalışmada, vinka alkaloidleri (vinblastin, vincristin, vinorelbin) ve paklitakselin DNA bağlanma aktivitelerinin araştırılması amaçlanmıştır.

GEREÇ VE YÖNTEM: İlaçların DNA ile etkileşimleri agaroz jel elektroforez deneyi ile analiz edildi. Her deneyde, 100 bp marker DNA, pUC19 plazmid DNA (2686 bp) ve pBR322 plazmid DNA (4361 bp) dahil olmak üzere üç tip DNA kullanıldı. DNA'lar belirli koşullar altında farklı ilaç konsantrasyonları ile inkübe edildikten sonra agaroz jel elektroforezi yapıldı. İlaç-DNA etkileşimlerinin yorumlanabilmesi için DNA bant dağılımları jel analiz sistemi ile analiz edildi.

BULGULAR: Sonuçlarımıza göre vinka alkaloidlerinden özellikle vinorelbinin, vinkristin ve vinblastine göre daha yüksek aktiviteyle DNA'ya bağlandığı bulunmuştur. Vinca alkaloidleri, DNA bağlanma aktivitesi için gerekli yapısal özelliklere sahiptir ve DNA bağlanma motiflerinde benzerlik vardır. Ancak sonuçlar, taksan grubundan olan paklitakselin DNA bağlama aktivitesine sahip olmadığını gösterdi. Bunun nedeni, paklitakselin kimyasal yapısının DNA'ya bağlanmaya uygun olmaması olabilir.

SONUÇ: İlaçların DNA ile interaksiyonu, ilaçların etki yollarını ve DNA hasarına neden olma yeteneklerini belirlemede önemli bir rol oynar. Sonuç olarak, çalışmamızın bulguları mikrotübül inhibitörü olan bu ilaçların etki mekanizmalarını ve genotoksik potansiyellerini aydınlatmaya katkı sağlayacaktır.

ANAHTAR KELİMELER: Anti-mikrotübül ilaçlar, Vinca alkaloidleri, Paklitaksel, DNA-bağlanma, Kanser terapi

ABSTRACT

OBJECTIVE: Microtubules are an essential part of the intracellular cytoskeletal structure and possess unique polymerization dynamics that are critical for many cellular functions, including cell division. Anti-microtubule drugs that interfere with microtubule formation are important chemotherapeutic agents for the treatment of various cancer. These drugs that block mitosis seem to work by a common mechanism, which suppresses the dynamics of microtubules, slows cells, induces apoptosis and subsequently kills tumor cells. Vinca alkaloids (vinblastine, vincristine and vinorelbine) and Taxanes (paclitaxel) are two different classes of anti-microtubule drugs that cause microtubule dysfunction and inhibit cancer cell proliferation. The main activity of vinca alkaloids and taxanes result from their binding interactions with tubulin proteins. However, studies on DNA interactions of these anti-microtubule drugs are not sufficient. In this study, it was aimed to investigate the DNA binding activities of the vinca alkaloids (vinblastine, vincristine, vinorelbine) and paclitaxel.

MATERIAL AND METHODS: The interactions of the drugs with DNA were analyzed by agarose gel electrophoresis assay. Three types of DNA were used in each experiment, including 100bp marker DNA, pUC19 plasmid DNA (2686 bp), and pBR322 plasmid DNA (4361 bp). After the DNAs were incubated with different concentrations of the drugs under certain conditions, agarose gel electrophoresis was performed. DNA band distributions were analyzed with a gel analysis system so that the drugs-DNA interactions could be interpreted.

RESULTS: According to our results, it was found that among the vinca alkaloids, especially vinorelbine binds to DNA with higher activity than vincristine and vinblastine. The Vinca alkaloids have structural properties required for DNA binding activity and there is a similarity in their DNA binding models. However, the results showed that paclitaxel, which is from the taxane group, did not have DNA binding activity. This may be because the chemical structure of paclitaxel is not suitable for binding to DNA.

CONCLUSIONS: The interaction of drugs with DNA play an important role in determining the pathways of drugs action and their ability to cause DNA damage. Consequently, the findings of our study will contribute to elucidating the effect mechanisms and the genotoxic potentials of these drugs, which are microtubule inhibitors.

KEYWORDS: Anti-microtubule drugs, Vinca alkaloids, Paclitaxel, DNA-binding, Cancer therapy

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INTRODUCTION

Chemotherapy constitutes one of the fastest growing areas of cancer biology and medical chemistry among traditional cancer treatment approaches (1). As cancer is considered a disease caused by a defect in the signal transduction pathways, oncology research is currently focused on elucidating cellular signaling pathways that result in programmed cell death (apoptosis). The long-term aim of cancer therapy is to kill malignant tumor cells by inhibiting some of the mechanisms mainly related to cell division (2 - 4). Microtubules are the main factors of usual cell cycle events such as mitotic spindle formation during cell division, preservation of cell shape, organelle localization, and integration of separated DNA. Due to these properties, they are an attractive target for anticancer drug design.

Microtubules are dynamic structures formed by the polymerization of α and β-tubulin proteins in eukaryotic cells. Microtubule dynamics play an important role in the mitosis process. During a long period of the cell cycle, microtubules form an intracellular lattice-like structure. However, when cells enter mitosis, this network of microtubules reorganizes into mitotic spindles. Depolymerizing the microtubule structure and recreating the mitotic spindle, as well as finding, linking, and separating chromosomes require highly coordinated microtubule dynamics (5, 6). Therefore, drugs that interfere with microtubule dynamics limit proliferation by inhibiting the ability of cells to complete mitosis successfully. These antimicrotubule drugs are very successful in cancer treatment and are recommended as the best known cancer targets (7 - 10). The success of these drugs is related to the mechanisms of action that lead to impairment of cell division and induction of apoptosis (11 - 13).

By far, Vinca alkaloids and taxanes are among the most clinically useful antimicrotubule drug classes. Both provide successful results in various human cancer treatments (14 - 16). Vinca alkaloids (e.g. vincristine, vinblastine, and vinorelbine) are cell cycle specific and block cells in the metaphase / anaphase transition of mitosis (15-17). Taxanes (e.g. paclitaxel and docetaxel) are potent inhibitors of cell proliferation and keep cells in mitosis, but in contrast to vinca alkaloids, they promote the polymerization of tubulin, causing stabilization and bundling of microtubules (16, 18, 19). Consequently, the target of both agent groups is tubulin / microtubule structure. Vinca alkaloids and taxanes interact with their specific cellular targets β-tubulin proteins, resulting in microtubule dynamic instability (10, 15, 16).

The drugs that inhibit the dynamic structure of microtubules have been used clinically in the treatment of various cancers for more than twenty years. These drugs bind to tubulin proteins and at high concentrations lead to a decrease or increase in microtubule mass between phases. Antimitotic drugs targeting the microtubule can be classified into two general groups.

The first group is microtubule-destabilizing agents that prevent microtubule polymerization and the vinca alkaloids (vincristine, vinblastine, vinorelbine) and similar compounds are included in this group. The second group includes microtubule-stabilizing agents. Unlike agents in the first group, they stimulate microtubule polymerization. This group includes agents such as paclitaxel (the first agent identified in this group), docetaxel. However, both groups of drugs have been shown to cause mitotic arrest and apoptosis by inhibiting mitosis due to the instability of the microtubule dynamic mechanism (15, 16).

Vinca alkaloids, isolated from *Catharanthus roseus* the periwinkle plant, are powerful microtubule-destabilizing agents. Vinblastine (VBL) and vincristine (VCR), the original members of this family, were introduced to the clinic long ago. Later, second generation semi-synthetic vinca analogs such as vinorelbine (VRL) and vinflunine (VFL) were developed. Chemically, vinca alkaloids possess dimeric chemical structures consisting of two basic polycyclic units, an indole nucleus (catarantin) and a dihydroindole nucleus (vindoline) combined with other complex systems (17, 20). Vinca alkaloids bind to the β -tubulin protein. This binding results in decreased microtubule dynamics and mitotic arrest (21). Different vinca alkaloids have unique pro-

perties. VBL inhibits angiogenesis and has been used clinically in the treatment of Hodgkin's disease, non-Hodgkin lymphoma and breast cancer. It has been shown that VRL has significant antitumor activity in breast cancer patients and induces anti-proliferative activity in osteosarcoma. VCR is widely used in the treatment of pediatric leukemias, hematological malignancies and some solid tumors (17, 20, 21).

Taxanes are the first-in-class microtubule stabilizing drugs. They stabilize the Guanosine diphosphate (GDP)-bound β-tubulin protofilaments by flattening them into a conformation similar to the more stable GTP-bound structure. Taxane binding causes the equilibrium heterodimers of tubulin to shift from the soluble form to the polymerized form, resulting in the bundling and stabilization of interphase microtubules. As a result, taxanes reduce microtubule dynamics and this results in abnormal mitotic spindle formation, mitotic arrest and induction of apoptosis (21). Paclitaxel (Taxol) is the first member of the taxane family used for cancer treatment. Originally isolated from the bark of the Pacific badger, *Taxus brevifolia*, paclitaxel is a complex diterpenoid that inhibits cell division and causes cell death. Paclitaxel and its semi-synthetic analogue docetaxel have become the mainstay in the treatment of solid tumors. Today, paclitaxel is one of the most important antitumor drugs and is widely used clinically in the treatment of breast, ovarian, and non-small cell lung cancer (16, 21, 22). The main activity profile of vinca alkaloids and taxanes results from their binding interactions with tubulin proteins. However, studies on DNA interactions of these anti-microtubule drugs are not sufficient. This study was aimed to investigate the DNA binding activity of the vinca alkaloids (vinblastine, vincristine, vinorelbine) and paclitaxel.

MATERIALS AND METHODS

Chemicals and reagents

The vinca alkaloids (VBL, VCR, VRL) and paclitaxel were obtained from Sigma Aldrich Co. USA. 100bp marker DNA, pUC19 and pBR322 plasmid DNA were purchased from Fermentas MBI Ltd. Tris, acetic acid, ethylenediamine-tetraacetic acid (EDTA), agarose, ethidium bromide and loading buffer were purchased from Sigma Aldrich Co. USA.

The Drug-DNA Binding studies

Initially, all the drugs were prepared in a 10 mM stock solution, each by initially dissolving in autoclaved milli-Q water. All solutions were stored at 4°C for testing in experiments.

The interactions of the drugs with DNA were analyzed by agarose gel electrophoresis assay. Three types of DNA were used in each experiment, including 100 bp marker DNA, pUC19 plasmid DNA (2686 bp) and pBR322 plasmid DNA (4361 bp). The reaction mixtures contained varied DNA types (1µg of 100 bp marker DNA, 0.5 µg of pUC19 DNA and 0.3 µg of pBR322 DNA), different concentrations of the antimicrotubule drugs and tris buffer 1M (pH 8.0) in a total volume 12µL. The mixtures were incubated for 1h at 37°C. After the incubation period, 3µL of loading buffer (0.25% bromophenol blue and 30% glycerol) was added to each the reaction mixture and then loaded into the wells of the gel in Tris-Acetate EDTA buffer (60 mM Tris, 30 mM Acetic acid and 1.5 mM EDTA, pH 8.0). The samples were subjected to electrophoresis on 2% agarose at 45 V for 3 h. After electrophoresis, the gels were stained with ethidium bromide (1 mg/mL), photographed under UV light and DNA band distributions were analyzed with a gel analysis system so that the drug-DNA interactions could be interpreted.

Ethical Commitee

In this study, laboratory experiments were conducted on completely commercially purchased products, so the study does not require ethics committee approval.

RESULTS

The results on the determination of DNA binding activities of the vinca alkaloids and paclitaxel by agarose gel electrophoresis are shown in Figures 1- 4 respectively.

In Figure 1, the binding of Vinorelbine to DNA was examined in different DNA samples. Drug–DNA complexes migrate more slowly than free DNA when subjected to agarose gel electrophoresis. The rate of DNA migration is retarded

when bound to the drug. As seen in Figure 1, it is clear that vinorelbine binds to DNA with high affinity. Because there is only pUC19 DNA in lane 5 while lanes 6 and 7 have pUC19 DNA + vinorelbine (5 and 2 µL doses respectively). As a result, it was very clearly seen that the sample containing pUC19 DNA + vinorelbine in lane 7 was far behind lane 5 (only pUC19 DNA) on the agarose gel. Although not as clear as in lane 7, similar results were observed in the loadings for 100 bp DNA and vinorelbine in lanes 1, 2 and 3, and for pBR322 DNA and vinorelbine in lanes 8 and 10. Because lanes 2 and 3 were seen slightly behind lane 1 and in a more faint state. A similar situation was observed for lanes 10 and 8 **(Figure 1)**.

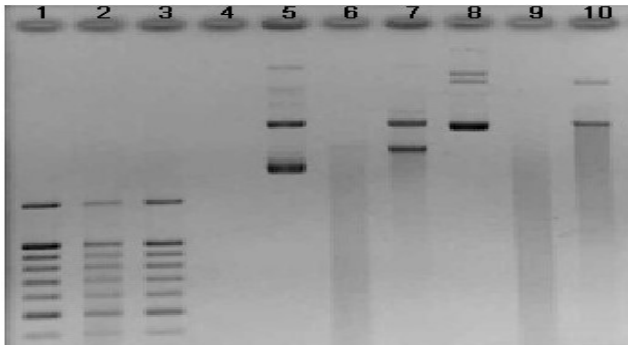

Figure 1: Vinorelbine-DNA Interaction
1. lane; 3µL 100 bp Marker DNA
2. lane; 3µL 100 bp Marker DNA+5µL Vinorelbine (10 mM Stock)
3. lane; 3µL 100 bp Marker DNA+2µL Vinorelbine (10 mM Stock)
4. lane; 3µL Vinorelbine (10 mM Stock)
5. lane; 0.5 µg pUC19 DNA
6. lane; 0.5 µg pUC19 DNA+5µL Vinorelbine (10 mM Stock)
7. lane; 0.5 µg pUC19 DNA+2µL Vinorelbine (10 mM Stock)
8. lane; 0.3 µg pBR322 DNA
9. lane; 0.3 µg pBR322 DNA+5µL Vinorelbine (10 mM Stock)
10. lane; 0.3 µg pBR322 DNA+2µL Vinorelbine (10mM Stock)

In Figure 2, the Vincristine-DNA interaction is shown. While lane 4 contains only pUC19 DNA, lanes 5-9 contain pUC19 DNA and different concentrations of vincristine, respectively. Similarly, lane 10 contains only pBR322 DNA while lanes 11-15 contain pBR322 DNA and different concentrations of vincristine, respectively. When agarose gel electrophoresis was completed, the interaction of vincristine with pUC19 DNA was not detected, whereas vincristine was observed to bind to pBR322 DNA. As a result, the sample containing pBR322 DNA + vincristine (5µL) in lane 11 appeared slightly behind lane 10 containing only pBR322 DNA on the agarose gel and as a more faint band **(Figure 2)**.

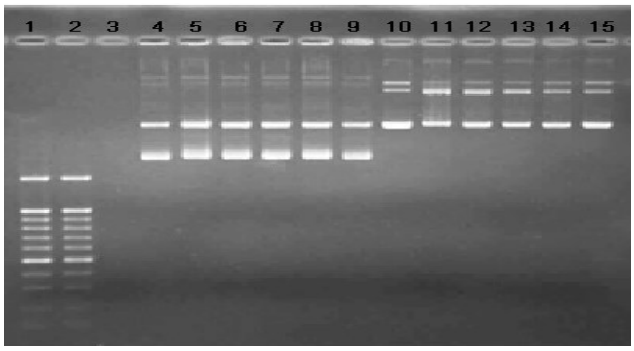

Figure 2: Vincristine-DNA Interaction
1. lane; 3µL 100 bp Marker DNA
2. lane; 3µL 100 bp Marker DNA+5µL Vincristine (10 mM Stock)
3. lane; 5µL Vinorelbine (10 mM Stock)
4. lane; 0.5 µg pUC19 DNA
5. lane; 0.5 µg pUC19 DNA+5µL Vincristine (10 mM Stock)
6. lane; 0.5 µg pUC19 DNA+2µL Vincristine (10 mM Stock)
7. lane; 0.5 µg pUC19 DNA+1µL Vincristine (10 mM Stock)
8. lane; 0.5 µg pUC19 DNA+1µL Vincristine (10 mM Stock 1/2 dilution)
9. lane; 0.5 µg pUC19 DNA+1µL Vincristine (10 mM Stock 1/4 dilution)
10. lane; 0.3 µg pBR322 DNA
11. lane; 0.3 µg pBR322 DNA+5µL Vincristine (10 mM Stock)
12. lane; 0.3 µg pBR322 DNA+2µL Vincristine (10 mM Stock)
13. lane; 0.3 µg pBR322 DNA+1µL Vincristine (10 mM Stock)
14. lane; 0.3 µg pBR322 DNA+1µL Vincristine (10 mM Stock 1/2 dilution)
15. lane; 0.3 µg pBR322 DNA+1µL Vincristine (10 mM Stock 1/4 dilution)

Figure 3 shows the Vinblastine-DNA interaction. Lane 4 contains only pUC19 DNA, while lanes 5-9 contain pUC19 DNA and different vinblastine concentrations, respectively. Similarly, lane 10 contains only pBR322 DNA while lanes 11-15 contain pBR322 DNA and different concentrations of vinblastine, respectively. The conformation of most plasmid DNAs is largely in the form of a supercoil. The conformation of the pUC19 and pBR322 plasmid DNAs which we used in our experiments, also is in the form of a large number of supercoils. However, if two chains of this double-stranded supercoil form are broken, it turns into a linear form, and if one chain is broken and the other chain remains circular, it turns into an open circular form. Thus, when plasmid DNAs are run in agarose gel, the supercoil form is seen the foremost, the linear form behind it, and the open circular form at the rearmost. When we evaluate in terms of only the supercoiled form which is the foremost visible in agarose gel, it was unclear the binding of vinblastine with pUC19 DNA because the foremost supercoil DNAs (lanes 4, 5, 6, 7, 8, and 9) appeared to have almost all run equally in the gel. However, a band appeared behind the foremost supercoil band in lanes 6 and 7 (pUC19 DNA + vinblastine 2µL and 1µL respectively),

these bands are absent in lane 4 (only pUC19 DNA). It is very likely that vinblastine (at doses of 2 and 1 µL) interacted with pUC19 plasmid DNA, breaking the two strands of the double-stranded supercoil pUC19 DNA converted it from the supercoiled form to the linear form (Figure 3).

When we evaluate the binding of vinblastine with pBR322 DNA in terms of only the supercoiled form seen in agarose gel, it can be said that vinblastine binds to pBR322 DNA. Because the sample containing pBR322 DNA + vinblastine (5µL) in lane 11 was slightly behind lane 10 containing only pBR322 DNA in agarose gel (Figure 3). At the same time, a band appeared behind the foremost supercoil band in lanes 12 and 13 (pBR322 DNA + vinblastine 2µL and 1µL respectively) , these bands are absent in lane 10 (only pBR322 DNA). Most likely, vinblastine (at doses of 2 and 1 µL) linked to pBR322 DNA, broke the two strands of the double-stranded supercoiled pBR322 DNA and transformed it from the supercoiled form to the linear form **(Figure 3)**.

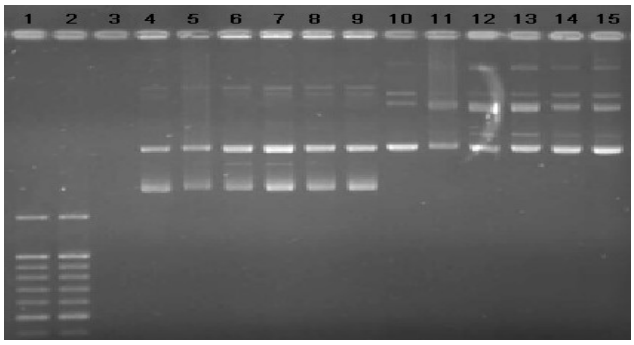

Figure 3: Vinblastine-DNA Interaction
1. lane; 3µL 100 bp Marker DNA
2. lane; 3µL 100 bp Marker DNA+5µL Vinblastine (10 mM Stock)
3. lane; 5µL Vinblastine (10 mM Stock)
4. lane; 0.5 µg pUC19 DNA
5. lane; 0.5 µg pUC19 DNA+5µL Vinblastine (10 mM Stock)
6. lane; 0.5 µg pUC19 DNA+2µL Vinblastine (10 mM Stock)
7. lane; 0.5 µg pUC19 DNA+1µL Vinblastine (10 mM Stock)
8. lane; 0.5 µg pUC19 DNA+1µL Vinblastine (10 mM Stock 1/2 dilution)
9. lane; 0.5 µg pUC19 DNA+1µL Vinblastine (10 mM Stock 1/4 dilution)
10. lane; 0.3 µg pBR322 DNA
11. lane; 0.3 µg pBR322 DNA+5µL Vinblastine (10 mM Stock)
12. lane; 0.3 µg pBR322 DNA+2µL Vinblastine (10 mM Stock)
13. lane; 0.3 µg pBR322 DNA+1µL Vinblastine (10 mM Stock)
14. lane; 0.3 µg pBR322 DNA+1µL Vinblastine (10 mM Stock 1/2 dilution)
15. lane; 0.3 µg pBR322 DNA+1µL Vinblastine (10 mM Stock 1/4 dilution)

Paclitaxel-DNA interaction is demonstrated in Figure 4. Lane 1 contains only 100 bp marker DNA, while lanes 2-3 contain 100 bp marker DNA and paclitaxel. Lane 5 contains only pUC19 DNA, while lane 6 contains pUC19 DNA and paclitaxel (2µL). Similarly, lane 7 contains only pBR322 DNA while lane 8 contains pBR322 DNA and paclitaxel (2µL). The experimental results showed that paclitaxel did not bind to DNA. Because it was observed that all DNA-paclitaxel samples moved at the same speed on agarose gel electrophoresis with lanes containing only DNA **(Figure 4)**.

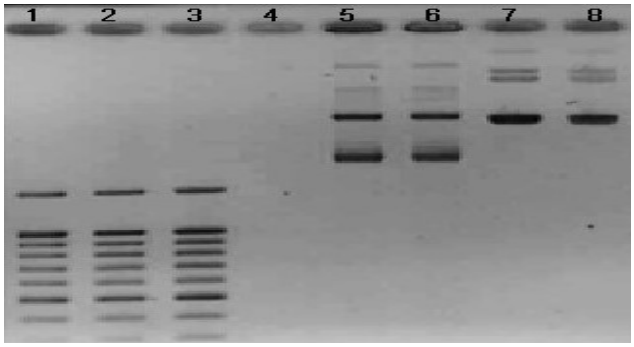

Figure 4: Paclitaxel-DNA Interaction
1. lane; 3µL 100 bp Marker DNA
2. lane; 3µL 100 bp Marker DNA+2µL Paclitaxel (10 mM Stock)
3. lane; 5µL 100 bp Marker DNA+2µL Paclitaxel (10 mM Stock)
4. lane; 3µL Paclitaxel (10 mM Stock)
5. lane; 0.5 µg pUC19 DNA
6. lane; 0.5 µg pUC19 DNA+2µL Paclitaxel (10 mM Stock)
7. lane; 0.3 µg pBR322 DNA
8. lane; 0.3 µg pBR322 DNA+2µL Paclitaxel (10 mM Stock)

DISCUSSION

Vinca alkaloids are an important class of alkaloids with wide chemotherapeutic potential. They are indole-based alkaloids with one or two indole rings in their structure. Vinblastine and vincristine are structurally similar, but vinblastine contains a methyl group while vincristine contains an aldehydic function attached to the nitrogen of the central indole moiety. This structural difference causes significant differences in both antitumor activity and toxicity of these agents (17, 20, 21). The main activity of vincristine and vinblastine alkaloids is due to reversible binding interactions with tubulin protein.

They depolymerize the microtubular assembly, thereby stopping cell division and causing cell death. Like other vinca alkaloids, vinorelbine also inhibits cellular proliferation by binding to tubulin, but differs from them according to its antitumor activity spectrum (16, 20, 21).

Paclitaxel, which is in the taxanes class, is among the most specific and successful chemotherapeutic drugs used in the treatment of breast and ovarian cancer (15, 16, 22). Paclitaxel has antimitotic properties resulting from the binding to tubulin and the over-stabilization

of microtubules. The mechanisms of action of taxanes differ significantly from vinca alkaloids in terms of pharmacology, clinical indications and toxicology. Structurally, paclitaxel is a complex ester composed of a 15-member taxane ring system linked to an unusual four-membered oxane ring.

In this study, DNA binding activities of the vinca alkaloids (vinblastine, vincristine, vinorelbine) and paclitaxel were investigated. According to our results, it was found that among the vinca alkaloids, especially vinorelbine binds to DNA with higher activity than vincristine and vinblastine. However, it was observed that paclitaxel, which is from the taxanes group, did not have DNA binding activity.

The results of this study showed that vinca alkaloids bind with DNA. This is due to a possible similarity in their DNA binding motifs. Vinca alkaloids have properties such as several H-bond acceptor / donor atoms, planar ring systems, and a large aromatic skeleton required for DNA binding activity (23). According to our results, it was found that among vinca alkaloids, especially vinorelbine bind to DNA with higher activity than vincristine and vinblastine. These findings showed that the affinity order for DNA binding was Vinorelbine> Vinblastine> Vincristine (Figures 1, 3 and 2, respectively).

In a study previously conducted by Mohammadgholi (2013) et al., the binding effect of vincristine on DNA and chromatin structure was examined using advanced spectroscopy techniques. It has been reported that vincristine binds with a higher affinity to chromatin than DNA, and shows higher binding affinity to double-stranded DNA compared to that single-stranded. Additionally, it has been declared that the vincristine-DNA interaction occurs through intercalation with binding to the phosphate sugar backbone and that histone proteins play a major role in this process (24).

The binding of vincristine changes the chromatin structure in some way, disrupting the histone-DNA interaction, and possibly the removal/displacement of histones from DNA. It is suggested that vincristine binds to the DNA molecule via H-bonding with DNA bases and sugar-phosphate backbone, as well as intercalation between bases. In addition, vincristine can enter the head domain of histones through hydrophobic interaction with the vindoline and catarantin domains (24). In our study, as a result of the paclitaxel-DNA interaction experiment, it was determined that there was no significant change in DNA band distributions compared to only DNA containing lanes, and therefore paclitaxel did not have DNA binding activity (Figure 4). This may be because of the chemical structure of paclitaxel that is not suitable for binding to DNA. Most chemotherapeutic drugs may show high binding affinities toward DNA bases and backbone. DNA interactions with antitumor drugs can result in various types of DNA damage. Therefore, understanding the mechanisms of drug–DNA binding is important for predicting their potential genotoxicity (25, 26). Furthermore, drug–DNA binding may result in changes in gene expression and affect cell proliferation (27). Eventually, the elucidation of drug-DNA binding mechanisms is critical for predicting the results of these interactions (28).

In summary, the results of our study indicate that among the vinca alkaloids, especially vinorelbine binds to DNA with higher activity than vincristine and vinblastine, while paclitaxel has no DNA binding activity. The interaction of drugs with DNA is a significant feature in pharmacology and plays a crucial role in the determination of the mechanisms of drugs action and their genotoxicity. In conclusion, the findings of our study will contribute to elucidating the effect mechanisms and the genotoxic potentials of these drugs, which are microtubule inhibitors. However, the results obtained need to be confirmed by more advanced analysis.

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