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Evaluation of the Proliferative Effects of Hexagonal Boron Nitride Nanoparticles on Leukemia Cells and Leukemia Stem Cells

Altıgen Bor Nitrür Nanopartiküllerinin Lösemi Hücreleri ve Lösemi Kök Hücreleri Üzerindeki Proliferatif Etkilerinin Değerlendirilmesi

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Abstract: Leukemia is a malignant disease that affects the bone marrow, lymphatic system, spleen, and blood-forming organs, leading to an excessive proliferation of white blood cells. Current cancer treatments are often limited by drug resistance, highlighting the need for novel therapeutic strategies. Nanoparticles, including boron nitride (BN) nanomaterials, have shown promise in enhancing drug delivery and therapeutic efficacy due to their excellent physical and chemical properties. This study aimed to evaluate the cytotoxic effects of hexagonal boron nitride nanoparticles (hBN NPs) on leukemia cells and leukemia stem cells to explore their potential use in leukemia treatment.: hBN NPs were synthesized and characterized using X-ray powder diffraction (XRD), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM). Leukemia cell lines (HL-60 and CCRF-CEM) and CD34+ leukemia stem cells were treated with various hBN NPs. Cell viability was assessed using MTS assays, and flow cytometry was employed to analyze the expression of leukemia surface markers. The study found that hBN NPs did not exhibit significant anticancer properties; instead, they promoted cell proliferation in leukemia cells and stem cells. The CCRF-CEM CD34+ cells showed resistance to hBN NPs treatment, which reduced the treatment's therapeutic efficacy. The lack of cytotoxicity toward healthy cells suggests potential selectivity, yet the proliferative effects on leukemia cells indicate that hBN NPs may not be suitable for leukemia treatment. hBN NPs lack therapeutic potential for leukemia due to their proliferative effects on leukemia cells. Future studies should focus on developing combination therapies and exploring hBN NPs' impact on other cell lines to identify potential synergistic strategies that could overcome resistance mechanisms in leukemia and other cancers.
Keywords: Leukemia, Hexagonal Boron Nitride Nanoparticles, Cytotoxicity

Özet: Lösemi, kemik iliğini, lenf sistemini, dalağı ve kan oluşturan organları etkileyen ve beyaz kan hücrelerinin aşırı çoğalmasına yol açan kötü huylu bir hastalıktır. Mevcut kanser tedavileri genellikle ilaç direnciyle sınırlıdır ve bu da yeni tedavi stratejilerine olan ihtiyacı vurgulamaktadır. Bor nitrür (BN) nanomalzemeleri de dahil olmak üzere nanopartiküller, mükemmel fiziksel ve kimyasal özellikleri nedeniyle ilaç iletimini ve tedavi edici etkinliği artırmada umut vadetmektedir. Bu çalışma, lösemi tedavisinde potansiyel kullanımlarını araştırmak için hekzagonal bor nitrür nanopartiküllerinin (hBN NP'leri) lösemi hücreleri ve lösemi kök hücreleri üzerindeki sitotoksik etkilerini değerlendirmeyi amaçlamaktadır. hBN NP'leri, X-ışını toz kırınımı (XRD), Taramalı Elektron Mikroskobu (SEM) ve Transmisyon Elektron Mikroskobu (TEM) kullanılarak sentezlendi ve karakterize edildi. Lösemi hücre hatları (HL-60 ve CCRF-CEM) ve CD34+ lösemi kök hücreleri çeşitli hBN NP konsantrasyonlarıyla tedavi edildi. Hücre canlılığı MTS analizleri kullanılarak değerlendirildi ve lösemi yüzey belirteçlerinin ekspresyonunu analiz etmek için akış sitometrisi kullanıldı. Çalışma, hBN NP'lerinin önemli antikanser özellikleri göstermediğini; bunun yerine lösemi hücrelerinde ve kök hücrelerinde hücre çoğalmasını teşvik ettiğini buldu. CCRF-CEM CD34+ hücreleri hBN NP tedavisine direnç gösterdi ve bu da tedavinin terapötik etkinliğini azalttı. Sağlıklı hücrelere karşı sitotoksisitenin olmaması potansiyel seçiciliği düşündürmektedir, ancak lösemi hücreleri üzerindeki çoğaltıcı etkiler hBN NP'lerinin lösemi tedavisi için uygun olmayabileceğini göstermektedir. hBN NP'leri lösemi hücreleri üzerindeki çoğaltıcı etkileri nedeniyle lösemi için terapötik potansiyele sahip değildir. Gelecekteki çalışmalar, lösemi ve diğer kanserlerde direnç mekanizmalarının üstesinden gelebilecek potansiyel sinerjistik stratejileri belirlemek için kombinasyon tedavileri geliştirmeye ve hBN NP'lerinin diğer hücre hatları üzerindeki etkisini araştırmaya odaklanmalıdır.
Anahtar Kelimeler: Lösemi, Hekzagonal Bor Nitrür Nanopartikülleri, Sitotoksisite

Ethics Committee Approval: Since this study is an in vitro cell culture study, ethics committee approval is not required. The cell lines used in the study were purchased from the relevant company.

Informed Consent: This study did not require informed consent.

Authorship Contributions: NM formulated the study designs, devised the experiments, and gathered as well as analyzed the data. EK ve FK supported the experimental stages and contributed to analyzing the data.

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1. Introduction

Leukemia is a malignant disease primarily affecting the bone marrow, lymphatic system, spleen, and blood-forming organs. It causes an excessive proliferation of a type of leukocyte, specifically white blood cells (WBCs), leading to leukocytosis (1). Leukemia presents in various forms, some more common in younger patients, while others predominantly affect adults. Although the exact etiology of leukemia remains unknown, several factors, including genetic predisposition, chromosomal abnormalities, chemical agents (such as benzene), chemotherapeutic drugs, radiation, immunodeficiency, and viruses, may contribute to the disease's development (2)

The complex structure of the tumor microenvironment and individual differences between patients make it even more difficult to develop effective cancer treatments. Therefore, research and development of new drug strategies are encouraged.

In recent years, intensive research has been conducted on nanoparticles and this technology has an important place among drug delivery strategies. Nanoparticle-based drug delivery systems have offered significant advantages in cancer treatment and management by providing excellent pharmacokinetic properties, precise targeting, reduced side effects, and superiority against drug resistance. The use of these systems has significant potential in developing more effective cancer interventions (3,4)

The use of nanoparticles (NPs) and nanocarriers in cancer therapy has significantly enhanced the delivery of chemotherapeutic agents, primarily by reducing their toxicity to healthy tissues. NPs offer several advantages, including improved bioavailability, enhanced solubility, extended blood circulation time, and minimized side effects. Furthermore, nano-delivery systems that incorporate targeting or sensing mechanisms have been shown to enhance the efficacy of anti-tumor drug candidates by enabling the selective release of therapeutics at specific target sites (5–7)

Among NPs, boron nitride (BN) nanomaterials have attracted considerable interest due to their excellent physical and chemical properties (8–10). Owing to its high biocompatibility, BN has shown significant potential in drug delivery and cancer treatment applications (11,12). Additionally, BN has been utilized in boron neutron capture therapy (BNCT) for tumor treatment due to its rich 10B content (13–16). However, the surface hydrophobicity of boron nitride nanomaterials facilitates them (17–19)

Cancer stem cells (CSCs) are inherently heterogeneous and exhibit low abundance within tumor populations, which poses challenges for their detection (20,21). Recent advancements in nanotechnology and the development of nanoparticles (NPs) have opened new avenues for the diagnosis and treatment of CSCs, enhancing the precision and effectiveness of therapeutic strategies.

The effects of boron nitride (BN) nanoparticles on leukemia remain largely unknown. To address this gap, our study investigated the cytotoxic effects of newly synthesized hexagonal boron nitride nanoparticles (hBN NP) on leukemia cells and leukemia stem cells, with detailed structural and morphological characterizations performed on the nanoparticles.

2. Materials and Methods

2.1.Synthesis and Characterization of Hexagonal Boron Nitride (hBN) Nanoparticles:

hBN NP was synthesized by reacting boron oxide with ammonia gas, followed by milling in a planetary ball mill and sieving under 150 microns to achieve high crystallinity. In the preparation method, sieving below 150 microns was used, but in the analysis results, the hBN NP size was determined to be 120 nm (22). Characterization was performed using X-ray powder diffraction (XRD) to confirm the crystalline structure, and Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) to analyze the morphology, revealing uniform hexagonal nanoparticles with well-defined features.

The hBN nanoparticles were characterized using imaging and spectroscopic techniques. Before SEM imaging, a conductive Au-Pd layer was sputtered for 40 seconds. SEM analysis revealed a uniform structure with lateral dimensions ranging from 50 to 200 nm and a generally round morphology. TEM imaging confirmed the hexagonal crystalline structure of boron nitride, with particle diameters within 50–200 nm and thicknesses between 15–50 nm. Parallel, straight-line crystalline features characteristic of hBN were observed, further verifying the structural uniformity of the nanoparticles.

Detailed structural and morphological characterization of the hBN nanoparticles used in this study, including SEM, TEM, and XRD analyses, have been previously published (22). The current study focuses on the biological effects of these well-characterized nanoparticles on leukemia cells.

2.2. Propagation of Leukemia Cell Line

HL-60 and CCRF-CEM cell lines obtained from ATCC® (American Type Culture Collection, Manassas, VA, USA) were maintained in RPMI-1640 medium supplemented with 20% fetal bovine serum, 2 mM l-glutamine, 1% PSA (10,000 units/ml penicillin and 10,000 µg/ml streptomycin, and 25 µg/ml of amphotericin B) for their proliferation and the cells were incubated at 37°C in a humidified atmosphere with 5% CO2. Cells were seeded into appropriate culture dishes according to their number and checked daily.

2.3. Obtaining 34+ Stem Cells from Leukemia Cell Lines

When the cells (CCRF-CEM) reached the desired density, they were collected by centrifugation. The cells were washed once with PBS. They were resuspended with PBS again and anti-human CD34+ fluorescently labeled antibodies (Biolegend, USA, Cat. No.343506), were used to selectively bind to CD34+ molecules on the cell surface. After 30 minutes of incubation in the dark, the cells were stained with DAPI. Afterwards, the gate was taken from the live cells and the necessary device adjustments were made and CD34+ cells were separated in the separation device. Before and after the separation in the flow cytometry device, CD34+ and other leukemia cancer markers were analyzed and the effect of the separation process was determined as a percentage (23).

2.4. Identification of CCRF-CEM CD34+ Leukemia Stem Cells Using Monoclonal Antibodies Targeting Leukemia Surface Markers

Following the isolation of CD34+ stem cells from CCRF-CEM cells using a flow cytometry sorting device, the quality of the isolated CCRF-CEM CD34+ cells was assessed by analyzing surface markers both before and after isolation, including CD34+ and other leukemia cancer surface markers (CD34, CD33, CD123, CD133). The impact of the isolation process was quantified as a percentage. The cells were stained with fluorochrome-conjugated antibodies following methodologies consistent with previous studies. For this purpose, leukemia cells were seeded at a density of 50,000 cells/well in a 96-well plate and labeled with leukemia surface antibodies according to the manufacturer's instructions (1:1000 dilution). The labeled cells were then analyzed by flow cytometry (23,24).

2.5. Peripheral Blood Mononuclear Cells (PBMNC)

Peripheral blood mononuclear cells (PBMNCs) isolated from healthy individuals (ATCC®, American Type Culture Collection, Manassas, VA, USA) were utilized as healthy controls. These PBMNCs serve as primary cells that can be directly compared to the leukemia cells under investigation, providing a physiologically relevant baseline for assessing differential cytotoxic effects between normal and cancerous cells. Observing the effects on healthy PBMNCs is critical to confirm that the treatment specifically targets leukemia cells without adversely affecting normal cells.

The isolation of healthy PBMNCs was performed using the classical Ficoll-Paque density gradient centrifugation method. To promote proliferation, PBMNC cultures were sustained in RPMI-1640 medium, enriched with 20% fetal bovine serum, 2 mM L-glutamine, and 1% PSA (comprising penicillin at 10,000 units/ml, streptomycin at 10,000 µg/ml, and amphotericin B at 25 µg/ml). Cultures were incubated at 37°C in a humidified environment with 5% CO_2.

2.6. Cell Cytotoxicity Test

The stock solution of hBN NPs was prepared by dissolving it in physiological serum and then diluted to the desired final concentrations with RPMI. 96 wells were seeded with RPMI medium (50 µL) supplemented with 10% FBS, 1% PSA (10,000 units/ml penicillin and 10,000 µg/ml streptomycin, and 25 µg/ml of Amphotericin B) at 5 x 10^3 cells per well. Then, the cells were treated with diluted concentrations of hBN NPs in four replicates. To determine the appropriate concentrations, initial doses of 10, 20, 40, 80, 160, and 320 µg were prepared, followed by a series of lower diluted concentrations at 2, 4, 6, 8, and 10 µg of hBN NPs. Samples not treated with hBN NPs were used as negative control and samples treated with DMSO 20% were used as positive control. After 24, 48, and 72 hours, MTS solution was added to each well according to the manufacturer's recommendations. MTS solution allows us to determine the number of living cells by causing a color change in the

presence of metabolically active cells that reduce the tetrazolium salt to a formazan dye. This reaction involves the conversion of the tetrazolium salt to formazan by the mitochondrial dehydrogenase activity of the cells. The cells were incubated in the dark at 37°C for 3 hours. The absorbance of the cells was measured at 490 nm. Analysis was performed according to the negative control.

2.7. Statistical Analysis

All data were statistically analyzed using one-way ANOVA or a two-tailed Student's t-test. GraphPad Prism (version 8.0.1) software was utilized for performing the statistical analyses and plotting the graphs. Error bars represent the standard deviation of the mean (SD) from a minimum of three independent experiments. Statistical significance was defined as *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, and ****P ≤ 0.0001.

3. Results

3.1. CCRF-CEM CD34+ Leukemia Stem Cells and Leukemia Surface Marker Analysis

Figure 1 illustrates the percentage of CCRF-CEM cells expressing leukemia-associated surface markers (CD34+, CD133+, CD123+, and CD33+) before and after isolation. Before isolation, the expression levels of these markers were generally low, with minimal percentages observed across all groups. Following isolation, there is a marked and statistically significant increase in the percentage of CD34+ cells, identified as Leukemia Stem Cells (LSCs), demonstrating successful enrichment of these cells (****p < 0.0001). Conversely, the percentages of CD133+, CD123+, and CD33+ cells remain relatively unchanged or low post-isolation, suggesting that the isolation process specifically enriches the CD34+ cell population without significantly altering the distribution of other markers.

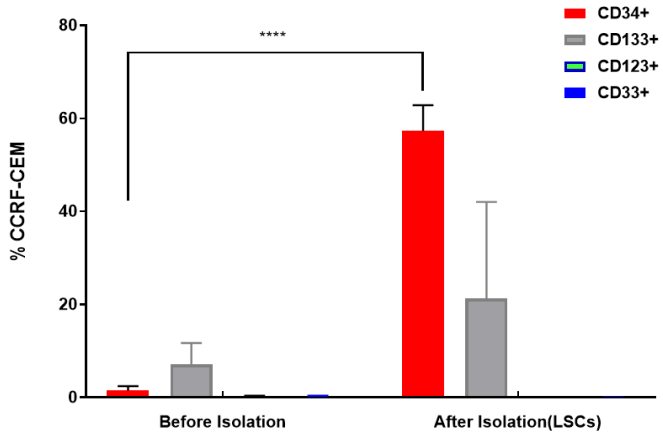

Figure 1. Percentage of CCRF-CEM cells before and after isolation (CCRF-CEM CD34+ cells considered as Leukemia Stem Cells, LSCs). The graph illustrates the expression levels of CD34+, CD133+, CD123+, and CD33+ markers in CCRF-CEM cells before isolation and after the enrichment of CD34+ cells. Statistical significance between the groups is indicated (****p < 0.0001).

3.2. Evaluation of Cellular Viability

Based on the data from Figure 2, the evaluation of CCRF-CEM CD34+ stem cell line showed that at low concentrations (10 - 40 µg) after 24 hours of treatment, cell viability increased compared to the control group, but the change was not statistically significant. After 48 hours of treatment, cell viability at these concentrations (10 - 40 µg) was significantly increased compared to the control (a: *p < 0.05, b: **p < 0.01). At 72 hours, cell viability also increased compared to the control group, with a statistically significant increase observed particularly at the 40 µg dose (b: **p < 0.01). At higher concentrations (80 - 320 µg), cell proliferation continued to increase across all time points. Specifically, at doses of 80 - 160 µg, significant increases in cell viability were observed at 48 and 72 hours compared to the control. At the highest concentration (160 µg), cell proliferation showed a statistically significant increase across all time points (c: ***p < 0.001, d: ****p < 0.0001).

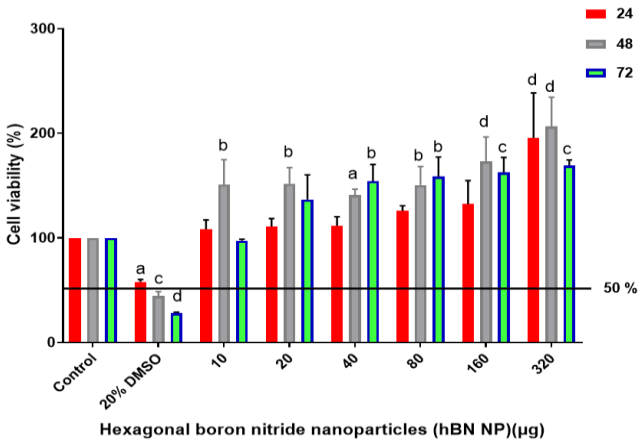

Figure 2. This bar graph illustrates the impact of various concentrations of hBN NP on CCRF-CEM 34+ cell viability, measured at different time points (24, 48, and 72 hours). The x-axis represents the doses of hBN NP used, given in microgram (µg) units, while the y-axis shows the percentage of cell viability compared to the control group. The error bars represent the standard deviation of the mean. The experiments were performed in triplicate. The horizontal dashed line indicates 50% cell viability. DMSO 20% was used as a positive control. Different concentrations are compared with the control group and significant differences are shown with other letters (a: *p < 0.05, b: **p < 0.01, c: ***p < 0.001, d: ****p < 0.0001).

Figure 3. represents the effects of hBN NP on HL-60 cells. After 24 hours of treatment, a statistically significant increase in cell proliferation was observed at all concentrations (a: *p < 0.05, d: ****p < 0.0001). Additionally, a significant increase in cell proliferation was noted at high doses (8 and 10 µg) of hBN NP after 48 and 72 hours of treatment compared to the control group (a: *p < 0.05, b: **p < 0.01). No significant changes were observed at other concentrations and time points.

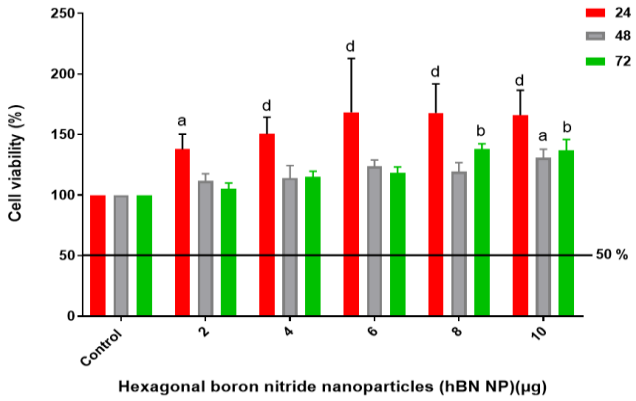

Figure 3. This bar graph illustrates the impact of various concentrations of hBN NP on HL-60 cell viability, measured at different time points (24, 48, and 72 hours). The x-axis represents the doses of hBN NP used, given in microgram (µg) units, while the y-axis shows the percentage of cell viability compared to the control group. The error bars represent the standard deviation of the mean. The experiments were performed in triplicate. The horizontal dashed line indicates 50% cell viability. Different concentrations are compared with the control group and significant differences are shown with other letters (a: *p < 0.05, b: **p < 0.01, d: ****p < 0.0001).

Figure 4. shows the effect of hBN NPs at various doses on the viability of healthy PBMNCs. A significant increase in cell viability was observed in healthy PBMNCs treated with hBN NPs at 6 and 10 µg concentrations for 48 hours compared to the control group (a: *p < 0.05, c: ***p < 0.001). No significant changes were observed at other concentrations and time points.

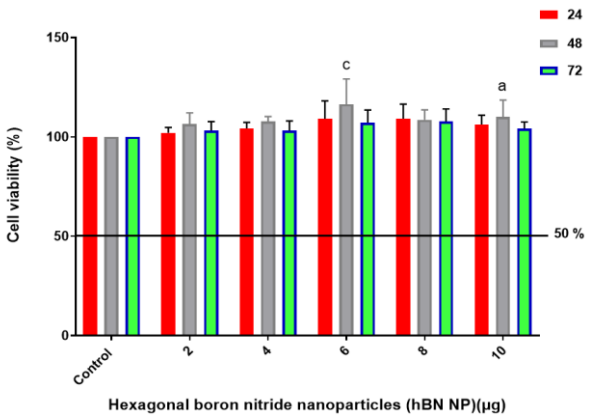

Figure 4. This bar graph illustrates the impact of various concentrations of hBN NP on healthy PBMNC viability, measured at different time points (24, 48, and 72 hours). The x-axis represents the doses of hBN NP used, given in microgram (µg) units, while the y-axis shows the percentage of cell viability compared to the control group. The error bars represent the standard deviation of the mean. The experiments were performed in triplicate. The horizontal dashed line indicates 50% cell viability. Different concentrations are compared with the control group and significant differences are shown with letters (a: *p < 0.05, c: ***p < 0.001).

4. Discussion

Identifying compounds that do not exhibit anticancer properties is critical for understanding the limitations of current cancer therapies and the underlying mechanisms of drug resistance. Studies have shown that various compounds are ineffective against cancer cells, highlighting the need for more targeted approaches in cancer treatment.

This study aimed to investigate the biological effects of hBN NPs on leukemia cancer cells and healthy cells to determine their potential therapeutic or adverse effects. The initial experiments focused solely on cell viability using MTS assays, which indicated that hBN NPs do not possess anticancer properties. As a result, no further investigations were pursued.

Leukemia stem cells are among the key factors influencing disease relapse and progression (25,26). Notably resistant to chemotherapy, these stem cells were exposed to high doses of hBN NPs. Despite the high dosage, a proliferative effect of hBN NPs was observed on CCRF-CEM CD34+ cells. One potential reason for this could be the activation of intracellular proliferation pathways, such as AKT/mTOR or MAPK signaling pathways, which may accelerate cell growth and division. One study found that one of the primary mechanisms by which boron affects cell proliferation is the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway. Studies have shown that low doses of boron can increase the proliferation of intestinal epithelial cells by activating this pathway, which is crucial for cell growth and survival (27). This activation is believed to occur through boron's estrogen-like effects, particularly via the estrogen receptor ERβ; this further modulates mitochondrial apoptosis signaling pathways, promoting cell proliferation while inhibiting apoptosis (27,28).

Boron-containing nanoparticles have been investigated for their ability to stimulate mesenchymal stem cell (MSC) proliferation and differentiation. In particular, boron-doped hydroxyapatites have been shown to increase MSC adhesion and promote osteogenic differentiation, which is critical for bone tissue engineering (29,30). The presence of boron in these nanomaterials not only improves cell viability but also regulates the expression of key proteins involved in bone formation, such as collagen and osteocalcin (31,32)

An additional mechanism potentially underlying the proliferative effects of hBN nanoparticles may involve their ability to mitigate intracellular oxidative stress, thereby enhancing cellular survival capacity. Additionally, the maintenance of cancer stem cell viability might be attributed to the suppression of apoptotic pathways and the upregulation of anti-apoptotic genes, such as BCL-2, by hBN NPs. Furthermore, hBN NPs might support cell growth through their effects on the extracellular matrix or cell microenvironment. These findings suggest that hBN NPs may lack potential therapeutic effects on leukemia stem cells.

The absence of the expected cytotoxic effect and the continued viability of CCRF-CEM CD34+ cells following high-dose application of hBN NPs suggest that the cells may have triggered adaptive or stress

responses to the excessive doses. In such scenarios, cells can exhibit an enhanced protective or proliferative response to high doses, possibly leading to adaptive mechanisms such as the activation of antioxidant defense systems or cell signaling pathways. Based on this observation, we transitioned to low-dose applications of hBN NPs to allow for a more precise evaluation of their effects and to elucidate the dose-response relationship in the cells more clearly. The rationale for using lower doses on HL-60 cells was to observe more physiological effects and to establish a more consistent dose-response curve. Additionally, since high doses may exhibit non-toxic or even stimulatory effects on cells, we hypothesized that the expected cytotoxic effect might be more pronounced at lower doses. Therefore, we aimed to more accurately assess the sensitivity of cancer cells to treatment and to identify potential therapeutic ranges. However, we observed that hBN NPs similarly exhibited a proliferative effect on HL-60 cells. Possible reasons for this outcome may include the enhancement of cell proliferation through the modulation of intracellular signaling pathways, such as PI3K/AKT and mTOR, the insufficient promotion or absence of ROS production, the increased expression of anti-apoptotic proteins (e.g., Bcl-2), or the reduction in the activity of pro-apoptotic proteins (e.g., Bax, Bak).

The interaction of boron nanoparticles with cellular pathways can also affect the expression of anti-apoptotic genes. Studies have shown that boron-containing compounds can induce the expression of genes that promote cell survival and inhibit those that lead to apoptosis (33). This dual effect promotes cell proliferation and increases cancer cells' resistance to therapeutic interventions. Additionally, hBN NPs might accelerate the cell cycle in leukemia cells by influencing proteins that regulate the cell cycle, such as cyclins and CDKs. Therefore, further research is needed to comprehensively study the biological effects of hBN NPs and assess their suitability for cancer treatment.

Consistent with our findings, a similar study demonstrated that pravastatin, a cholesterol-lowering drug, is ineffective in reducing the growth of neuroblastoma cells in culture. This observation aligns with previous research showing that open-ring statins like pravastatin do not exhibit significant anticancer activity, unlike their closed-ring counterparts (34). These results indicate that the structural characteristics of these compounds play a crucial role in their biological activity against cancer cells.

Moreover, drug resistance remains a significant obstacle in cancer treatment. It is well established that chemotherapy agents, such as paclitaxel, initially kill cancer cells but eventually become ineffective due to the development of chemotherapy resistance (35). This resistance can be attributed to various factors, including genetic mutations and alterations in drug metabolism, which limit the efficacy of many anticancer agents. The inability of some compounds to maintain effectiveness against evolving cancer cell populations underscores the importance of ongoing research into alternative therapeutic strategies.

The role of the tumor microenvironment in drug resistance cannot be overlooked. It has been noted that the microenvironment of solid tumors can hinder the penetration of anticancer drugs, thereby reducing their efficacy (36). This limitation is further complicated by the heterogeneity of cancer cell populations within tumors, which can exhibit varying sensitivities to treatment (37–39)

There is an increasing need for selective anticancer agents that can effectively target malignant cells without affecting normal cells (40). The narrow therapeutic window of many current treatments often results in significant side effects, highlighting the need to identify compounds that, although lacking inherent anticancer properties, can serve as adjuvants in combination therapies or enhance the efficacy of more potent agents. In our observations, hBN NPs did not demonstrate significant toxicity toward healthy cells, as evidenced by the lack of a meaningful reduction in cell viability. In cancer treatment, the ideal agents are those that can selectively target cancer cells while sparing healthy cells. The lack of toxicity of hBN NPs in healthy cells is crucial for potential therapeutic applications, as it suggests that the compound might affect only cancer cells while preserving healthy cells. The minimal toxicity of hBN NPs to healthy cells also implies a reduced inflammatory response, which could help prevent systemic toxicity and inflammatory complications. The biocompatible and biostable properties of hBN NPs may further support tissue regeneration and repair by interacting favorably with cells. Their ineffectiveness on healthy cells could be advantageous for maintaining tissue integrity. Given these positive attributes, further studies should explore the effects of hBN NPs on cancer cells, particularly in solid tumors, to

assess their broader therapeutic potential. Boron has also been shown to significantly affect cardiac myocytes. Studies have shown that boron increases DNA synthesis and facilitates cell cycle entry in cardiomyocytes, indicating its potential role in cardiac regeneration after injury (41). This regenerative capacity is further supported by findings that boron promotes the expression of growth factors and cytokines necessary for tissue repair and regeneration (29,42)

Boron also plays a role in immune cell proliferation. For example, it has been observed that boron can induce lymphocyte proliferation and regulate macrophage responses that are vital for immune function. The interaction of boron with certain biological ligands and stabilizing macromolecular complexes may contribute to these immunological effects by enhancing the overall immune response (43)

The findings suggest that boron-based hBN NPs exhibit proliferative effects on leukemia cancer cells and stem cells, indicating that they may not be suitable for leukemia treatment. Our results demonstrate that leukemia cancer cells develop resistance to hBN NPs treatment, which limits the impact of the treatment on the cells and reduces its therapeutic efficacy. Studies on leukemia and other resistant cancer types indicate that the reasons for the lack of response to treatment are associated with various factors, including genetic and epigenetic alterations, cell cycle regulation, disruption of apoptotic mechanisms, and non-drug-related mechanisms (44,45).

The use of hBN NPs in combination with other therapeutic agents could enable the development of novel treatment strategies that may alter the response of cancer cells to therapy. Combination therapies involving different nanoparticles or chemotherapeutic drugs can exhibit synergistic or additive effects in cancer cells, potentially targeting resistance mechanisms and enhancing therapeutic efficacy. Therefore, it is recommended to expand the scope of studies involving boron nanoparticles and test them on other cell lines. Future studies using various leukemia cell lines and stem cell models could provide insights into how and under what conditions resistance to treatment develops, guiding the development of targeted therapeutic strategies against these resistance mechanisms.

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