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Antitumor Activity of NMS-P937 Specific Small-Molecule Polo-Like Kinase 1 Inhibitor, in PC3 Human Prostate Cancer, Hela Cervical Cancer, and SKOV-3 Ovarian Cancer Cell Lines

PC3 Insan Prostat Kanseri, Hela Servikal Kanser ve SKOV-3 Over Kanseri Hücre Hatlarında, Spesifik Küçük Moleküllü Polo Benzeri Kinaz 1 Inhibitörü NMS-P937'nin Antitümör Aktivitesi

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ÖZ

Amaç: Spesifik küçük molekül polo benzeri kinaz 1 (PLK1) inhibitörü olan NMS-P937'nin PC3 insan prostat kanseri, HeLa servikal kanser ve SKOV-3 over kanseri hücre hatlarında antitümör aktivitesini araştırmayı amaçladık.
Araçlar ve Yöntem: PC3, HeLa ve SKOV-3 hücreleri, 48 saat boyunca NMS-P937 ile muamele edildi. Canlılık, XTT kolorimetrik tahlil ile analiz edildi ve PC3'ün en hassas hücre dizisi olduğu bulunduğundan, toplam oksidan status (TOS) değerleri, NMS-P937 ile tedavi edilmiş ve edilmemiş PC3 hücrelerinde TOS tahlili ile değerlendirildi.
Bulgular: Kanser hücre hatlarının proliferasyonu, konsantrasyondaki artışla bağlantılı olarak NMS-P937 tarafından orta derecede inhibe edildi. NMS-P937'nin PC3, HeLa ve SKOV-3 hücrelerindeki 48s IC_{50} değerleri 27.3, 69.7 ve 79.3 µM olarak kaydedildi. TOS, kontrol ve NMS-P937 ile muamele edilmiş PC3 hücrelerinde ölçüldü ve sırasıyla 3.15±0.36 ve 4.49±0.64 µmol H_2O_2 Equiv./L olarak hesaplandı, bu da çalışma bileşiğinin etkisi altında artan oksidatif stresi gösterdi (p=0.035).
Sonuç: PLK1 inhibitörü NMS-P937, PC3 insan prostat kanseri, HeLa servikal kanser ve SKOV-3 over kanserinden oluşan kanser hücre hatlarının aktivitesini doza bağlı bir şekilde azaltır. Bu bileşik oksidatif stresi arttırır ve bu da bileşiğin PC3 hücrelerinde sitotoksik aktivitesinde çok önemli bir rol oynayabilir. Ancak yine de farklı kanser hücre dizileri ve tümör modellerini içeren hem in vitro hem de in vivo çalışmaların yapılmasına ve gelişebilecek olumsuz etkilerin ortaya çıkarılmasına ihtiyaç vardır.

Anahtar Kelimeler: kanser hücre hatları; NMS-P937; oksidatif stress; PLK1 inhibisyonu; sitotoksisite

ABSTRACT

Purpose: We aimed to investigate the antitumor activity of NMS-P937, a specific small-molecule polo-like kinase 1 (PLK1) inhibitor, in PC3 human prostate cancer, HeLa cervical cancer, and SKOV-3 ovarian cancer cell lines.
Materials and methods: PC3, HeLa, and SKOV-3 cells were treated with NMS-P937 for 48 h. The viability was analyzed by XTT colorimetric assay. Since PC3 was found to be the most sensitive cell line, total oxidant status (TOS) values were evaluated in NMS-P937-treated and non-treated PC3 cells via TOS assay.
Results: The proliferation of cancer cell lines was moderately inhibited by NMS-P937 in conjunction with the increase in concentration. The IC_{50} values of NMS-P937 in PC3, HeLa, and SKOV-3 cells were recorded as 27.3, 69.7, and 79.3 µM, respectively, for 48 h. TOS was measured in control and NMS-P937-treated PC3 cells and calculated as 3.15±0.36 and 4.49±0.64 µmol H_2O_2 Equiv./L, respectively, indicating the increased oxidative stress under the influence of the study compound (p=0.035).
Conclusion: The PLK1 inhibitor NMS-P937 reduces the activity of cancer cell lines consisting of PC3 human prostate cancer, HeLa cervical cancer, and SKOV-3 ovarian cancer in a dose-dependent manner. This compound increases oxidative stress, which may play a pivotal role in the cytotoxic activity of the compound in PC3 cells. However, there is still a need to carry out both in vitro and in vivo studies, including different cancer cell lines and tumor models, and to reveal the adverse effects that may develop.

Keywords: cancer cell lines; cytotoxicity; NMS-P937; PLK1 inhibition; oxidative stress

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INTRODUCTION

Cancer is an important part of global health problems, and according to statistics from developing countries, death from heart disease is considered the second major disease.[1] In addition, the aging world population with the extension of human life and the increase in the number of aged people increase in cancer-causing habits such as smoking continues to increase cancer incidence.[1] Globally, one in five people suffer cancer in their lifespan, and one in eight men and one in eleven women die as a result. The aging of the world's population and socio-economic risk factors remain among the main factors contributing to this increase. Breast cancer is one out of every four women diagnosed worldwide. Cervical, thyroid, lung, and colorectal cancers are also common among women; prostate and lung cancers are the most common in men and they together account for almost one-third of all male cancers.[2] Based on a recent report from the American Cancer Society, over 248 thousand, 14 thousand, and 21 thousand new cancer cases of the prostate, cervix, and ovary, respectively, and over 34 thousand, 4 thousand, and 13 thousand deaths from cancers of the prostate, cervix, and ovary, respectively, are projected to occur in the United States in 2021.[3]

PLK family consists of five members (PLK1-PLK5), which are involved in centrosome ripening, control point retrieval, cytokinesis, spindle assembly, apoptosis, and numerous other features of cellular functions and cell division. In addition to the role of PLK1 in adapting DNA damage and restarting the cell cycle[4], recent results have come to the fore that PLKs are associated with tumor development and are overexpressed in many cancer cell types.[5]

The assembly of a bipolar spindle and centrosome maturation function occurs via PLKs. Micro-injection of PLK1-specific antibody into the cell is known to result in the disruption of cell division while causing the formation of abnormal chromatin and microtubules.[6] The size and number of centrosomes required to organize microtubules in the cell increase before mitosis begins.[7] The many protein aggregations, including M-phase-specific phosphoprotein (MPM-2) reactive phosphor epitopes, and the monitoring of γ-tubulin are indicative of the functional ripening of centrosomes. Much smaller mitotic spindle polarities were formed in cells injected with PLK1-specific antibody, and it was seen to reduce the expression of MPM-2 and γ-tubulin.[5,6]

Nowadays, drugs effective during mitosis, such as vinca alkaloids and taxanes, are among the most accomplished chemotherapeutics used in antineoplastic therapy. These medications are microtubule-binding compounds that hinder the mitotic spindle function to arrest the cell cycle in mitosis and encourage apoptosis in cancer cells. These chemotherapeutics not only affect proliferating cancer cells but also lead to critical side effects by affecting the intracellular carrying processes mediated by microtubules on non-proliferative cells, including neurons.[8]

Peripheral neuropathy is dose-limiting toxicity for agents whose mechanism of action is on microtubule binding, and at least two neuropathies have been reported in up to 30% of cancer patients using paclitaxel. As a result, there is a special interest in developing new antimitotic drugs that target non-microtubular structures.[9]

NMS-937, one of the phase II clinical trial compounds, is a PLK1 inhibitor with good selectivity and oral bioavailability.[10] NMS-P937, a selective small-molecule PLK1 inhibitor, is also suitable for both injection and oral administration.[10] We aimed to investigate the antitumor activity of NMS-P937 simultaneously in PC3 human prostate cancer, HeLa cervical cancer, and SKOV-3 ovarian cancer cell lines and to reveal its association with oxidative stress. Since the most NMS-P937-sensitive cell line was found to be PC3 cells, the treatment-related oxidant status was measured on PC3 cells.

MATERIALS and METHODS

Cell Lines and Culture Conditions

PC3 (CRL-1435), HeLa (CCL-2), and SKOV-3 (HTB-77) cell lines (American Type Culture Collection, ATCC, USA) were cultured in Dulbecco Modified Eagle's Medium (DMEM; Gibco Thermo Fisher Scientific) supplemented with 1% L-glutamine (Sigma-Aldrich), 1% penicillin-streptomycin (Sigma-Aldrich) and 10% fetal bovine serum (FBS; Sigma-Aldrich). The cell cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2.

Stock solution for NMS-P937 (Medchem) was prepared using dimethyl sulfoxide (DMSO; Sigma-Aldrich)[11] and further diluted with DMEM before administration to a final DMSO percentage less than 0.5%.

Cell Viability Assay

PC3, HeLa, and SKOV-3 cell lines were analyzed by XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carbox-anilide) colorimetric assay (Roche Diagnostic) to evaluate the anti-proliferative activity of NMS-P937.[12] Before the treatment was administered, the cells had been seeded in 96-well culture plates with a density of 1×10^4 cells/well in a 100-µL culture medium (DMEM) and incubated overnight. 1, 5, 10, 25, 50, and 100 µM final concentrations of NMS-P937 were then administered to the cells for 48 h. A mixture of 100 µL of DMEM culture medium (without phenol red) and 50-µL of XTT labeling solution was placed into each well to measure viable cells. Later, the plates were incubated at 37ºC for 4 h. After this incubation period, the plates were shaken, and the absorbance was measured at 450 nm with an ELISA microplate reader (Thermo). The cell viability was expressed as live-cell amount percent compared to the non-treated control. All experiments were conducted in triplicate, and the IC_{50} values of NMS-P937 in all the investigated cancer cell lines were determined by Graph Prism 7 software (GraphPad).

Measurement of TOS Values

PC3 cells, the most NMS-P937-responsive cell line compared to other cancer cell lines, were treated with NMS-P937 at the IC_{50} dose for 48 h. NMS-P937-untreated (control) and -treated PC3 cells were analyzed with the Total Oxidant Status Assay Kit (Rel Assay Diagnostics, Turkey) according to the manufacturer's protocol.[13]

Briefly, the kit standards and cell supernatants premixed with reaction reagent (reagent I) were applied in the wells. The staining reagent (reagent II) was then applied in the following process, and incubation was carried out at 37°C for 5 minutes. The absorbance was then read at 530 nm. With this method, which is based on the oxidation of ferrous ions to ferric ions in the presence of sufficient oxidant in the environment, TOS levels are tested by measuring ferric ions in xylenol orange samples. The assay results in which H_2O_2 was used to calibrate the assay were expressed as micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 Equiv/L).[13,14]

Statistical Analysis

Statistical analysis was performed by the Kruskal-Wallis ANOVA test with post-hoc Dunn's and Mann-Whitney tests as appropriate. The data were given as mean±standard deviation (SD) and a p-value less than 0.05 was considered statistically significant. Data analyses and graphic presentations were performed using GraphPad Prism 7.0 software (GraphPad, USA).

RESULTS

NMS-P937 Treatment Reduced the Cell Proliferation of Cancer Cells

This study demonstrated the cytotoxic activity of NMS-P937 in each of the multiple cancer cell lines consisting of PC3 human prostate cancer, HeLa cervical cancer, and SKOV-3 ovarian cancer. The growth of cancer cell lines was moderately inhibited by NMS-P937 in a concentration-dependent manner (Figure 1), and the IC_{50} values of NMS-P937 in PC3, HeLa, and SKOV-3 cells were noted to be 27.3, 69.7, and 79.3 µM for 48 h, respectively.

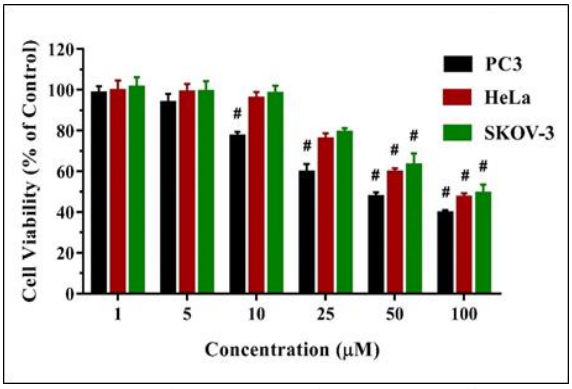

Figure 1. Antiproliferative activity of NMS-P937 at different concentrations (1-100 µM) on PC3, HeLa, and SKOV-3 cell lines. After 48 h of treatment, the cell viability was determined by XTT assay. All data are presented as mean ± SD in triplicate. #Denotes significant differences with the following p values. p=0.044 for 10 µM vs. control in PC3 cell line. p=0.037 for 25 µM vs. control in PC3 cell line. p=0.028 for 50 µM vs. control in PC3 cell line. p=0.021 for 100 µM vs. control in PC3 cell line. p=0.024 for 50 µM vs. control in HeLa cell line. p=0.018 for 100 µM vs. control in HeLa cell line. p=0.027 for 50 µM vs. control in SKOV-3 cell line. p=0.019 for 100 µM vs. control in SKOV-3 line.

Furthermore, since PC3 cells were found to be more sensitive to NMS-P937 compared to HeLa and SKOV-3 cell lines, a TOS assay was carried out within this cell line.

NMS-P937 Treatment Altered TOS in PC3 Cells

The effect of NMS-P937 on the TOS of PC3 cells was measured with a TOS assay kit.[13] As a result of the application of all doses to the cell lines, changes in oxidative stress in the PC3 cells were observed, but only the results of the experimental group in which the active substance was applied at a dose equal to the IC_{50} dose are shown in the article. PC3 cells were treated with NMS-P937 at the IC_{50} concentration for 48 h and the assay was performed. TOS values were calculated as 3.15±0.36 and 4.49±0.64 μmol H_2O_2 Equiv/L in control and NMS-P937-treated PC3 cells, respectively, indicating increased oxidative stress under the effect of the study compound (p=0.035) (Figure 2).

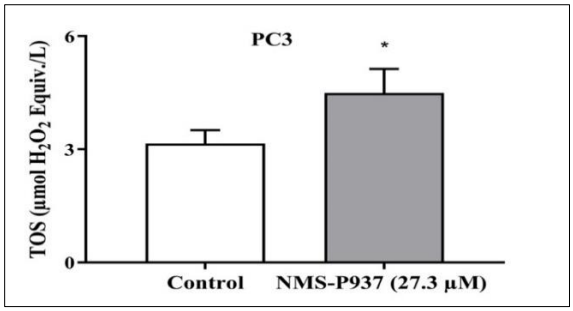

Figure 2. Determination of TOS values in PC3 cells. Treatment with the IC_{50} value of NMS-P937 for 48 h significantly increased TOS of PC3 cells compared to the untreated control. TOS was determined with the TOS assay kit, and the results were obtained as mean ± SD from three samples of the media from culture wells containing PC3 cells. *Significantly different compared to control (p=0.035).

DISCUSSION

This study demonstrated the anti-proliferative activity of NMS-P937 on PC3, HeLa, and SKOV-3 cell lines examined by XTT assay at different concentrations (1-100 μM) of NMS-P937 for 48 h for the first time. There was a dose-dependent decrease in the cell viability in all the cancer cell lines; however, this effect was significantly pronounced in the PC3 cell line ($p<0.05$). Additionally, in the PC3 cells exposed to 27.3 μM of NMS-P937 for 48 h, the treatment increased the TOS of PC3 cells. These results may indicate that the cytotoxic effect of NMS-P937 on PC3 is due to its induction of oxidative stress.

Despite approaches based on multiple anti-tumoral combinations or surgical downsizing, only modest improvement has been shown in the overall five-year survival rate of advanced cancer patients. Although traditional treatment approaches depend on the medical history and health status of the individuals, it is still important to develop less tiring but effective options in cancer control because side effects such as nausea, hair loss, weakness, and fatigue are quite discomforting.[15]

A serine/threonine-protein kinase PLK1 plays an essential role in the cell cycle regulation by intervening in steps such as cytokinesis, bipolar spindle formation, chromosome separation, and centrosome maturation that occur during mitosis.[12] Overexpression of PLK1, one of five identified mammalian homologs, is believed to cause weakening in checkpoints leading to excessive cellular division and thus cancer, including solid tumors, lymphomas, and leukemia. This overexpression linked to cancer progression, chemotherapeutic drug resistance, and poor prognosis makes PLK1 inhibition an attractive strategy for anticancer therapy.[10,16]

PLK1 is considered an encouraging target that provides an advantage to developmental studies of anticancer drugs, which can be used as monotherapy or in combination with other chemotherapies. Although a lot of effort has been made in clinical trials for PLK1 inhibitors, none of them have yet been licensed by the FDA.[17]

Therefore, it is vital to conduct detailed preclinical studies to determine which malignancies will benefit from PLK1 inhibitors and what doses of PLK1 inhibitors influence malignancies. Among ongoing drug studies to arrest the cell cycle and induce apoptosis in cancer cell lines and xenograft tumor models through depletion or inhibition of PLK1 activity, NMS-P937 (also known as MNS-1286937, onvansertib) is emerging as a novel inhibitor specific to PLK1.[18] A total of six studies were found, four in progress and one completed, for NMS-1286937 OR Onvansertib OR PCM-075 in ClinicaTtrials.gov search conducted in the preparation phase of this article.

Although remarkable efforts have been made over the decades, major challenges remain their significance in the treatment of prostate cancer, cervical cancer, and ovarian

cancer. Understanding the cellular and molecular abnormalities involved in these cancers with a multi-disciplinary approach will be insightful for targeted therapies and enhancement of clinical outcomes. Considering the role and importance of PLK1 inhibitors in cancer treatment, in this study, the antitumor activity of NMS-P937, a potential therapeutic, was evaluated in three different cell lines consisting of the prostate, cervical and ovarian cancers.

Prostate cancer is believed to be triggered by the interrelation of many pathways, including androgen receptor (AR) signaling and oxidative stress. Zhang et al. demonstrated that oxidative stress activates signaling in prostate cells in a Plk1-dependent manner.[19] Li et al. showed the sensitivity to treatment protocol in 22RV1, a BRCA1-deficient castration-resistant prostate cancer cell line, with Plk1 inhibitor combination, as well as in castration-resistant prostate cancer xenograft tumors.[20] Xu et al. developed a therapy that had a dual effect on both BET and PLK1 inhibitions.[21] They achieved anti-proliferation activity and induced apoptosis on castration-resistant prostate cancer cells in vitro and tumor growth suppression with the castration-resistant prostate cancer xenograft model in vivo.

Trial studies of PLK1 inhibitors in the treatment of cervical cancer continue with great speed and promising results. The overexpression of PLK1 in cervical cancer and its clinical implications have shed light on treatment modalities over PLK1, demonstrating the promising possibility of PLK1 as a biological marker for cervical cancer. In a recent publication, the issue has gained importance with sufficient evidence of PLK1 overexpression in cervical cancer.[22] Guo et al. also supported our study by concluding that PLK1 knockdown inhibited the growth of HeLa cervical cancer cells.[23]

PLK1 activation, a well-recognized oncogene outlined in many cellular processes, has been implicated in the oncogenic role of Aurora Borealis in ovarian cancer.[24] In another study investigating the factors affecting the proliferation and migration of cancer in SKOV3 and RMG1 ovarian cancer cell lines with transwell assay, flow cytometry, and methyl thiazole tetrazolium (MTT), the importance of regulation of PLK1 expression on the proliferation and migration of cancer cell lines was emphasized.[25] In a study involving stages I/II ovarian cancer patients, after demonstrating that high PLK1 expression is associated with poor prognosis, triple combination (paclitaxel/the potent PLK1 inhibitor BI6727/proTAME) induced pronounced apoptosis in ovarian cancer cell lines, OVCAR-3 and SKOV-3, and primary ovarian cells derived from cancer patients.[26] The authors compared the viability of OVCAR-3 and SKOV-3 cells after individual and combination therapies to consider the prognostic role and therapeutic potential of PLK1 for ovarian cancer. They emphasized that the combinatorial approach with a PLK1 inhibitor and proTAME is a promising strategy to efficiently lower the IC_{50} of paclitaxel in ovarian cancer cells. This triple treatment revealed important results for developing PLK1-included combinatorial treatments.

Compared to the IC_{50} value in the SKOV-3 ovarian cancer cell line in our study, Affatato et al. showed that lower concentrations of PLK1 inhibitors are active in mucinous and non-mucinous ovarian cancer cell lines (serous cell lines), including SKOV3.[27] However, there are reports hypothesizing that at high concentrations, PLK1 inhibitors might promote mitotic slippage events that promote cell survival.[28] Therefore, there remains a need to assess different doses of PLK1 inhibitors in various cell lines. Studies evaluating these comparisons, such as the content of our study, are important as they form the basis for future in vivo experiments.

As the role of PLK1 in the cancer mechanism is clarified, PLK1 inhibitors also qualify for potential in the clinical management of other cancers.[29] NMS-P937 decreased the clonogenic and migration ability of human osteosarcoma cell lines.[29] NMS-P937 has been proven to be an anticancer therapeutic against acute myelogenous leukemia (AML) in different experimental preclinical studies; furthermore, this agent has demonstrated a good oral bioavailability in combination with another antineoplastic agent Cytarabine, and promised long-term survival.[30] Casolaro et al. demonstrated a model of AML as AML-NS8 in mice by injection of leukemic blasts from CD56+ monoblastic AML (M5a) patient with the aggressive phenotype.[31] NMS-P937 displayed cytotoxicity to AML-NS8 cells with IC_{50} of 36 nM and showed promising results

compared to standard therapies with increased median survival time, making the opinion that it can support therapeutic use in AML. NMS-P937 was attributed as a candidate to have an important place in combination therapies, referring to the survival rates of combination therapy with cytarabine in animals with a disseminated model of AML, and tumor regression results of combination therapy with irinotecan in HT29-human colon adenocarcinoma xenografts.[10] A recently published phase Ib study of orally administered onvansertib in a 28-day cycle was conducted in relapsed or refractory AML patients in combination with either low-dose decitabine or cytarabine. Dose-limiting toxicity, maximum tolerated dose, pharmacokinetics, safety, and antileukemic activity were investigated within the study.[32] The results of this study, in which the combination of decitabine and onvansertib was well tolerated and antileukemic activity was recorded and provided useful information for further studies.

Our findings indicate that PLK1 inhibitor NMS-P937 diminishes the activity of cancer cell lines, including PC-3 human prostate, HeLa cervical cancer, SKOV-3 ovarian cancer, in a dose-dependent manner. PLK1 inhibition promises hope for monotherapy or combinatorial therapy strategies in cancer. We believe that MNS-P937 can be highly effective in combating cancer and other PLK1-related diseases. However, there is still a need to plan both in vitro and in vivo studies, including different cancer cell lines and tumor models, and to reveal the adverse effects that may develop.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Ethics Committee Permission

Ethics committee approval is not required for this study.

Authors' Contributions

Concept/Design: NY. Data Collection and/or Processing: NY. Data analysis and interpretation: NY, AG. Literature Search: AG. Drafting manuscript: AG. Critical revision of manuscript: AG.

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