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Cytotoxic and genotoxic effects of nateglinide on human ovarian, prostate, and colon cancer cell lines

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Abstract

Aim: Nateglinide, an oral anti-diabetic medication used to treat type 2 diabetes, activates ATP-dependent potassium channels in pancreatic beta cells and induces insulin secretion. Numerous antidiabetic medicines, particularly metformin, are known to drastically reduce the viability of cancer cells. This study examined the effects of nateglinide on the DNA and viability of human ovarian (A2780), prostate (LNCaP), and colon (Caco-2) cancer cells.

Materials and Methods: Initially in the study, 1, 10, 100, and 1000 µM doses of nateglinide were administered for 24 hours to A2780, LNCaP, and Caco-2 cells. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was used to measure cell viability. Using Graphpad Prism 8, the inhibitory logarithmic concentration values ($LogIC_{50}$) of nateglinide in A2780, LNCaP, and Caco-2 cells were computed based on the results of the MTT experiment. These doses were applied to A2780, LNCaP, and Caco-2 cells for the Comet assay. The Bonferroni-corrected Mann–Whitney U test was used to compare groups, and a value of $p<0.05$ was considered statistically significant.

Results: In A2780 and LNCaP cell lines, only 1000 µM nateglinide concentration decreased cell viability ($p<0.05$), whereas in Caco-2 cells, all concentrations except 1 µM reduced cell viability ($p<0.05$). The Comet assay indicated that nateglinide produced DNA damage by increasing the tail lengths and tail moments of A2780, LNCaP, and Caco-2 cells ($p<0.05$) and reducing the head diameters ($p<0.05$).

Conclusion: According to the findings of this study, nateglinide has cytotoxic effects on human ovarian, prostate and colon cancer cell lines and may possess anticancer properties.

Introduction

Cancer is formed as a result of uncontrolled self-replication of DNA and abnormal cell proliferation; it is the second disease with the greatest global fatality rate [1]. In 2020, the World Health Organization reported 19.2 million cases and 9.9 million deaths worldwide [2]. Cancer is a significant public health issue in our country, since it is the second leading cause of mortality following cardiovascular system diseases on the list of known causes of death [3]. Ovarian cancer is one of the most lethal forms of gynaecological cancers in the world [4]. The high death rate of ovarian cancer has made this form of cancer intriguing and treatments such as surgery and radiotherapy have been utilised frequently. Although various treatment strategies have been attempted for years to cure this form of cancer, there has been little success in increasing the patient survival rate [5]. On the other hand, prostate cancer is one of the leading causes of cancer-related death in males; it is the second most commonly diagnosed form of cancer worldwide [6]. In 2020, prostate cancer caused 375.000 deaths globally [2]. Based on previous research, it is believed that prostate cancer will become a major public health concern in the near future [7]. Colon cancer, one of the most prevalent digestive system cancers, accounts for 10% of male and female cancer cases [8]. Colon cancer has been responsible for 935.000 deaths worldwide in 2020 [2]. Chemotherapy is a cancer treatment method in which various chemotherapeutic agents are utilized to destroy cancerous cells. However, resistance to pharmaceuticals used to inactivate cancer cells and side effects caused by chemotherapeutic agents negatively impact the patient's daily life. In order to effectively cure cancer, it is crucial for the scientific community to discover novel drugs with minimal or no adverse effects and great selectivity [9].

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Metformin, a biguanide derivative, is a commonly utilized antidiabetic drug. Metformin, which inhibits gluconeogenesis and stimulates glucose uptake in skeletal muscles, is currently one of the medications of choice for treating type 2 diabetes. [10, 11]. Literature demonstrates that the drug's therapeutic effects are not limited to the treatment of diabetes mellitus, but also play a role in the treatment of numerous other disorders [11-13]. According to epidemiological studies, metformin positively affected the prognosis of cancer patients and inhibited tumour formation [14, 15]. Similar to metformin, nateglinide is an oral anti-diabetic medication used to treat type 2 diabetes. Nateglinide is an amino acid derivative of D-phenylalanine, one of the essential amino acids, and it induces rapid and short-term insulin production by altering the ATP potassium channels in the beta cells of the pancreas. It possesses a high level of reliability and tolerance. It's transitory and selective influence on short-term insulin secretion is insufficient to induce hypoglycemia. The small intestine absorbs nateglinide rapidly and completely, with an estimated bioavailability of 72%. The medication is extensively processed by the liver and is highly bound to plasma proteins. In several clinical trials, nateglinide has been proven to be safe, effective, and well-tolerated, both alone and in combination with oral anti-diabetic medications [16, 17]. When taken before a meal, nateglinide controls postprandial blood glucose effectively. Rapid nateglinide activity on pancreatic beta cells promotes and restores the initial phase of insulin secretion. Consequently, it is known to lower the blood glucose level after a meal [18]. In a retrospective study analyzing the association between nateglinide and cancer, it was believed that nateglinide prevents colorectal cancer [19]. In addition to this, there are few research examining nateglinide's influence on cancer in the literature. The purpose of this study was to investigate the cytotoxic and genotoxic effects of nateglinide on human ovarian (A2780), prostate (LNCaP) and colon (Caco-2) cancer cell lines.

Materials and Methods

Cell culture

In this study, the A2780, LNCaP and Caco-2 cell lines were utilized. All cell lines were cultured and prepared for the experiment in 75 cm^2 culture flasks. A2780 and LNCaP cell lines were cultured in RPMI-1640 medium (Sigma-Aldrich, USA; made by adding 10% Fetal Bovine Serum (FBS), 100 U/ml penicillin, and 0.1 mg/mL streptomycin), whereas Caco-2 cells were cultured in DMEM F-12 medium (10% FBS, 100 U/ml penicillin, and 1 ml insulin). Confluent cells cultivated in a carbondioxide (5% CO_2) incubator (ESCO, Singapore) at 37°C were extracted from flasks using trypsin-EDTA (Sigma-Aldrich, USA), and the viability of the cells was assessed by staining them with 0.4% trypan blue. Experimental studies were conducted on cell lines with a viability of at least 90% [20, 21].

Table 1. Calculated $LogIC_{50}$ (µM) values of nateglinide for A2780, LNCaP and Caco-2 cell lines.

Nateglinide (µM)	A2780	LNCaP	Caco-2
$LogIC_{50}$	2.506	2.814	1.226

MTT assay

To test the cytotoxic effects of nateglinide, confluent cells were counted by removing them from flasks using a trypsin-EDTA solution and transferred to 96-well well plates containing $15x10^3$ cells per well. The inoculated cells were allowed to attach to the plate base after a 24-hour (37°C, 5% CO_2). Following incubation, four doses of nateglinide (1, 10, 100, and 1000 µM) were applied to the wells containing cells, which were then incubated for 24 hours at 37°C in a CO_2 incubator. The 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT) technique was used to assess the cytotoxic effect of the test substance on A2780, LNCaP, and Caco-2 cell lines. First, a sterile MTT solution containing 0.5 mg/ml was produced in phosphate buffer. After treating cells with compounds, the medium was removed from each well of the plates, 50 µl of the prepared MTT solution was added to each well, and the plates were incubated for 3 hours in a CO_2 incubator. At the conclusion of this period, the MTT solution was withdrawn from the wells, 100 µl of DMSO was added to each well, and the optical densities of the cells in each well were measured using an ELISA plate reader (Thermo MultiskanGo, USA) at 550 nm [22]. This value was recognized as 100% viable cells based on the average of the absorbance readings obtained by reading the control wells (wells containing just media). The receptor values obtained from nateglinide-treated wells were compared to the absorbance value of the control well, and the % viability values were determined [23-25]. These experiments were conducted at least ten times separately on various days.

Calculation of $LogIC_{50}$ values

Based on the MTT experiment results for nateglinide concentrations of 1, 10, 100, and 1000 µM, the $LogIC_{50}$ (Inhibition Concentration 50) was computed. $LogIC_{50}$ is the inhibitory concentration that reduces cell viability by 50%. Graphpad Prism 8 was utilised to perform this calculation.

Comet assay

Comet assay, also referred to as single cell gel electrophoresis, is commonly used to detect DNA damage (Genotoxicity) in mammals [26]. Minor modifications were made to the Neutral Comet assay procedure described by Devlin et al. [27]. First, the grinding slides were coated with 0.65% high melting agarose (HMA) dissolved in PBS (Phosphate Buffer Saline) and allowed to dry in the dark for 1 day. A2780, LNCaP, and Caco-2 cells were cultured with various doses of the test compound (1, 10, 100, and 1000 µM) for the amount of hours determined by the $LogIC_{50}$ values. After incubation, the cells were mixed with low melting agarose at 42 °C and spread on an HMA-coated slide. The slides were then rapidly covered with a coverslip and stored in the dark at +4 °C for 10 to 15 minutes until the agar hardened. The slides were then put in a freshly made cold lysis solution from the stock lysis solution (created by adding 1% Triton X-100 and 10% DMSO) (2.5 M

Table 2. TL, TI and OTM values 24 hours after administration of nateglinide to A2780, LNCaP and Caco-2 cell lines (p*<0.05).

		Tail Lenght	Tail Intensity	Olive Tail Moment
A2780	Control	2770.00±895.07	15.87±9.66	520.01±295.00
	Solvent (DMSO)	2933.33±681.85	20.13±9.45	588.82±286.71
	1 µM	3861.53±941.09*	28.91±11.56*	976.62±633.36*
	10 µM	4111.11±980.32*	48.69±10.35*	1168.08±359.22*
	100 µM	3836.36±587.60*	42.34±13.92*	950.12±350.78*
	1000 µM	3900.00±1009.51*	47.41±19.06*	1215.52±597.48*
LNCaP	Control	2573.91±475.98	53.51±21.17	373.00±180.80
	Solvent (DMSO)	2991.66±517.44	53.94±19.03	350.05±126.58
	1 µM	3390.00±1705.06*	70.07±24.46*	647.54±427.51*
	10 µM	5905.26±4971.19*	149.97±104.07*	1262.52±746.48*
	100 µM	17492.30±6116.44*	690.89±449.40*	7261.88±4821.70*
	1000 µM	18257.14±9800.61*	1053.30±569.87*	6566.31±4784.68*
Caco-2	Control	2381.81±678.67	56.38±24.84	307.24±123.98
	Solvent (DMSO)	4069.56±1895.07	124.67±81.05	684.34±498.71
	1 µM	18083.33±11578.49*	507.39±424.78*	4966.43±4249.62*
	10 µM	7652.63±4427.61*	194.35±138.34	1399.05±1129.59*
	100 µM	7133.33±4866.21*	189.27±177.23	1432.81±1394.92
	1000 µM	8222.22±4780.29*	186.52±127.87	1434.84±1051.16*

NaCl, 100 mM EDTA, 10 mM Tris, pH:10). again in the dark at +4 °C for 1 hour. After the treatments, the cells were observed using a Leica fluorescent microscope (Figure 2), and the level of DNA damage was determined using Comet IV software. At least 25 cells from each slide were counted at random to calculate the tail lengths (TL), tail densities (TI), and olive tail moments (OTM) parameters of the groups (Table 2). Changes in TL, TI, and OTM parameters allowed us to determine DNA damage presence and rate.

Statistical analysis of data

IBM SPSS Statistics 24.0 (Windows) was utilised for the analysis. When statistically significant differences were found between the groups, multiple comparisons were conducted using the Mann Whitney U test with Bonferroni correction (all values of p<0.05 were considered statistically significant).

Results

In vitro cytotoxic activity

Figure 1A depicts the percentage changes in cell viability rates after A2780 cells were treated with nateglinide at varied doses (1, 10, 100, and 1000 µM) for 24 hours. Figure 1B demonstrates that a 1000 µM concentration of nateglinide decreased the viability of A2780 cells (p<0.05). The 1000 µM concentration of nateglinide was observed to impair cell viability in LNCaP cells (p<0.05). After incubating Caco-2 cells with varying doses of nateglinide (1, 10, 100 and 1000 M) for 24 hours, cell viability rates were observed. The resulting percentage changes are shown in Figure 1C. It was determined that 10, 100, and 1000 µM doses of nateglinide lowered the viability of Caco-2 cells (p<0.05). Table 1 presents the $LogIC_{50}$ values for A2780, LNCaP and Caco-2 cells based on the MTT assay findings of nateglinide for 24 hours. Using the obtained $LogIC_{50}$ values for all cell types, it was established that at the lowest concentration, nateglinide killed 50% of Caco-2 cells.

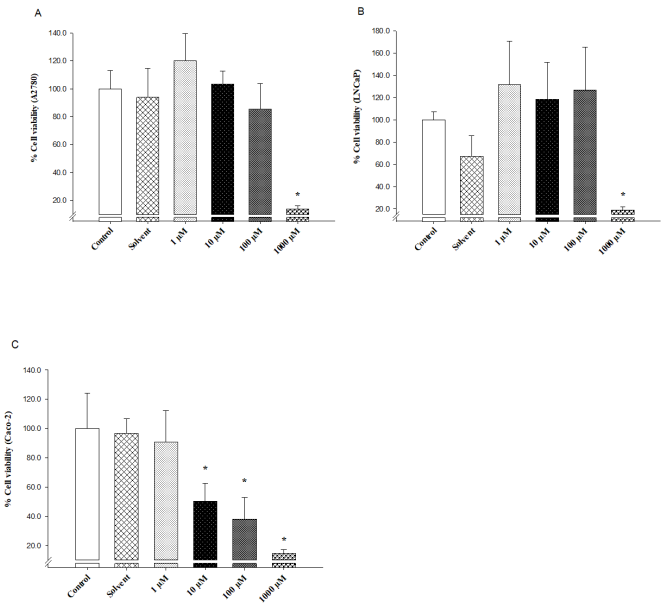

Figure 1. Cell viability of A2780 (A), LNCaP (B) and Caco-2 (C) cancer cell lines after nateglinide administration (*p<0.05).

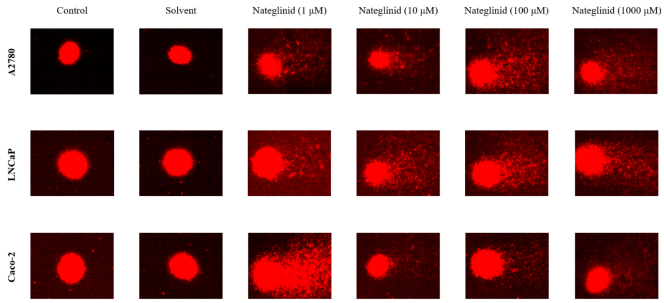

Figure 2. Images obtained from three different cancer cells in mhich nateglinide was effective in Comet assay trials.

In vitro genotoxic activity

In this study, we investigated whether DNA damage mediates the cytotoxic effects of nateglinide on ovarian, prostate, and colon cancer cell lines. All concentrations of human ovarian, prostate and colon cancer cell lines were examined for DNA damage. As a consequence of the investigation, TL, TI, and OTM parameters for all concentrations of A2780, LNCaP, and Caco-2 cell lines were determined, as well as the presence and rate of DNA damage (Table 2). In addition, images of the Comet assay are shown in Figure 2.

Discussion

Cancer is one of the major causes of death worldwide and an increasingly prevalent health concern [28]. Traditional cancer treatments, including surgery, chemotherapy and radiation therapy, as well as the recently developed immune therapy for cancer prevention, are used to eradicate cancer cells or inhibit their proliferation. These cancer treatments extend the life expectancy of cancer patients, although the majority of patients face recurrence issues. Consequently, present treatment methods appear to be a temporary solution [29]. Although research in the field of cancer is increasing, treatment methods impose a significant socioeconomic burden on countries and impose financial and moral constraints on individuals. Traditional types of treatment, such as radiotherapy and surgery, negatively impact the life of patients [30]. Although the primary objective of treatment is to prevent the abnormal proliferation of cancer cells, to neutralize these cells, and to activate the immune system mechanisms of individuals, the majority of chemotherapeutic drugs used to treat cancer have been linked to significant side effects with signs of acute and chronic toxicity [31, 32]. Serious adverse effects related to therapy are documented, especially in the gastrointestinal, excretory, and blood systems [33]. For this reason, research into alternative drugs/agents for the treatment of many forms of cancer continues. In recent years, the consumption of cancer-preventative oral medications has increased. Oral medicines have become the therapy of choice for the majority of cancer types due to their anticancer properties and forms designed to prevent genetic problems [34]. Recent research has discovered a connection between antidiabetic medicines and the prevalence of cancer. Some of the studied drugs have been demonstrated to lessen the chance of tumour growth, according to studies. A significant portion of the research focuses on the effects of metformin in the treatment of type 2 diabetes. According to research on breast, pancreatic, and liver cancer, this medicine may have a carcinogenic effect. In several research, it has been hypothesised that diabetes mellitus and cancer are diseases that can coexist, and according to the results of recent investigations, anti-diabetic and anti-cancer drugs may have carcinogenic effects on some organs [35]. In addition, Dąbrowski M. stated in his study [36] that antidiabetic drugs can modulate cancer risk by directly influencing the metabolism of cancer cells and indirectly influencing malignancy risk variables. It has been observed that nateglinide improves endothelial function and lipid profile, decreases oxidative stress, platelet activity and inflammatory markers, and slows the evolution of carotid intima-media thickness [37]. Similarly, Wang J. et al. [38] underlined that nateglinide strongly suppressed IL-1 secretion in their investigation. In light of these findings, it was hypothesised that nateglinide, an anti-diabetic medicine with anti-inflammatory properties, may be connected with cancer. Nateglinide, an amino acid derivative of D-phenylalanine, was observed in our study to significantly reduce the viability of A2780, LNCaP and Caco-2 cell lines. 1000 µM dosages of the drug in A2780 and LNCaP cells; It was determined that doses of 10, 100, and 1000 µM caused significant reductions in Caco-2 cell viability. The fact that there were changes in TL, TI, and OTM parameters (Table 2), and that these changes were statistically significant, indicated that cellular death due to DNA damage could be the cause of the decrease in cell viability (Figure 2, $p<0.05$).

All of these findings indicated that Nateglinide may have anticancer and anti-inflammatory properties. The fact that the cancer cells used in the study are human-specific enhances the significance of the study's findings. It is crucial to determine how this drug will perform in in vivo experiments and what impact it will have on healthy tissues.

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Ethical approval

Ethical approval was not required as it was a cell culture study.

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