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ORIGINAL ARTICLE

EFFECTS OF NICKEL CHLORIDE ON CELL MORPHOLOGY AND MIGRATION IN NON-SMALL CELL LUNG CANCER CELL LINES

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ABSTRACT

Aims: The discovery of the anti-cancer properties of the metal-based compound cisplatin and its effective use in cancer treatment has opened the door to the investigation of the anti-cancer properties of many other metals and metal-based compounds. Studies have shown that nickel chloride ($NiCl_2$) could suppress cell migration and metastasis in some types of cancer and could even be a promising anti-cancer agent in oral cancers, although the activity of $NiCl_2$ on the cell morphology and cell migration in non-small cell lung cancer cell lines (A549) is unknown. Thus, we aimed to investigate the role of $NiCl_2$ on cell morphology and migration in non-small cell lung cancer cell lines.

Methods: The present study investigates the effect of $NiCl_2$ on cell morphology and cell migration in A549 cell lines using a Giemsa staining technique, with an *in vitro* scratch analysis performed to determine the effect of $NiCl_2$ on cell migration.

Results: No significant change was observed in cell morphology in the group treated with 200 µM $NiCl_2$ compared to the control group, while the cellular morphology was changed in the cell lines treated with 600 µM $NiCl_2$. The cells lost cell-to-cell contacts, the cytoplasm shrank, and their morphology diverged from that of their ancestors, taking on a spindle-shaped and more unhealthy appearance. In addition, it was observed that cell confluency was decreased by half. It was found that $NiCl_2$ treatment at a dose at which cell morphology changed (600 µM) significantly reduced cell migration after 12 hours, and the effect was sustained at 24 and 48 hours, with cell migration significantly suppressed.

Conclusion: Our results suggested that treatment of non-small cell lung cancer cell lines with $NiCl_2$ changed cell morphology in a dose-dependent manner and suppressed the migration of cancer cells.

Keywords: Neoplasms, therapeutics, lung neoplasms, nickel

INTRODUCTION

Nickel (Ni), the 28th element in the periodic table, is a hard and ductile transition metal that is silvery-white in color (1). It has several oxidative forms (from -1 to +4), and the +2 oxidative form (Ni2+) is the most common form in the environment and biological systems (2). In general, Ni exposure most frequently occurs through the oral route in water and nutrients (3). Ni exposure can also occur through skin contact and inhalation (4). Ni, defined as immunotoxic and cancerogenic depending on the dose and duration of exposure, can cause various health problems such as contact dermatitis, cardiovascular disease, asthma, pulmonary fibrosis, and respiratory tract irritation (5). Soluble and insoluble Ni were identified as human carcinogens by the International Agency for Research on Cancer based on these toxic effects (6).

Nickel chloride ($NiCl_2$), a Ni compound, has been demonstrated to be a very weak carcinogen, producing no tumor after intramuscular injection in rats, and the administration of $NiCl_2$ alone did not cause skin tumors in mice, although studies have reported carcinogenic effects of elementary Ni and Ni salts (7). $NiCl_2$ has been defined as a non-genotoxic carcinogen as it does not directly cause changes in DNA, although the mechanisms underlying $NiCl_2$-induced cancer development have yet to be elucidated (8).

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There have been many recent studies reporting that Ni chloride suppresses cellular proliferation and induces cell death in some cancer types, and these characteristics could indicate anticancer properties (9, 10). Moreover, the treatment of $NiCl_2$ in some cancer types has been shown to suppress metastasis, and because of these effects, it has been suggested that $NiCl_2$ may be a new promising anticancer agent for the treatment of oral cancer. Although studies have demonstrated the efficacy of $NiCl_2$ against cell migration and cellular morphology in some cancer cell lines, there is no evidence of the effects of $NiCl_2$ on non-small cell lung cancer cell lines (9).

This study investigates the effects of $NiCl_2$ on cellular morphology and cell migration in non-small cell lung cancer cell lines.

MATERIAL AND METHODS

Cell Culture

The human non-small cell lung cancer cell lines (A549) used in the present study were purchased from the commercial American-type cell culture collection (ATCC, CCL-185). The cells were cultured at 37 °C in a humidified incubator with 5% CO_2 in a DMEM/F12 (Sigma, Cat. No: D6421) medium containing 10% fetal bovine serum (Fetal Bovine Serum, Biological Industries, Cat. No: 01-121-1A), 2 mM L-Glutamine (Biological Industries, Cat. No: 03-020-1B) and 100 µg/mL penicillin/streptomycin (Biological Industries, Cat. No: 03-031-1B).

Giemsa Staining

Giemsa staining was used to determine the effect of $NiCl_2$ on the cell morphology in A549 cell lines. The cells were seeded in 96-well culture plates, with each well containing 5,000 cells. The cells were treated with $NiCl_2$ for 24 hours at doses of 200 µM and 600 µM, as determined in previous preliminary studies (10-12). The mediums were discarded at the end of the specified period, and the cells were washed once with phosphate buffered saline (PBS). The cells were then incubated with PBS/methanol at a rate of 1:1 for two minutes. The content of the wells was discarded, and the cells were incubated with fresh methanol for 10 minutes, then the methanol was discarded and the cells were incubated for two minutes after adding Giemsa staining to the wells. The Giemsa stain was removed, and the wells were washed with distilled water for two minutes. The morphology of cells was examined under a light microscope.

Cell Migration Analysis

An *in vitro* scratch analysis was used to evaluate the effects of $NiCl_2$ on cell migration in A549 cell lines. The A549 cells were seeded in 96-well culture plates, each well containing $2x10^4$ cells. After 24 hours, a 200 µL pipet was used to scratch the well in the middle from one end to the other to create an artificial wound. The cells were treated with 600 µM $NiCl_2$. The area between the two ends of the wound was calculated at 0, 6, 12, 24, and 48 hours to evaluate the degree of cell migration. Migration areas were calculated using ImageJ 1.53 software.

Statistical Analysis

All data were processed using GraphPad Prism 8.0 statistical software. Differences between control groups and nickel chloride treated groups were analyzed using Student's t-test. p values less than 0.05 (*), 0.01 (**), and 0.001 (***) were considered statistically significant.

RESULTS

Treatment with Nickel Chloride Affects A549 Cell Morphology in a Dose-dependent Manner

Giemsa staining was used to determine the effect of $NiCl_2$ on the cellular morphology of A549 cell lines for which the A549 cell lines were subjected to $NiCl_2$ at doses of 200 µM and 600 µM for 24 hours. At the end of this period, the changes in cellular morphology were observed by examining the $NiCl_2$-treated groups in comparison with the group not treated with $NiCl_2$ under a light microscope. The microscopic examination revealed no significant changes in cellular morphology in the group treated with a dose of 200 µM compared to the control group, while the cellular morphology changed in the cell lines treated with 600 µM $NiCl_2$ with a loss of cell-to-cell contact, a decrease in the cytoplasm, and a morphological divergence from their ancestors in gaining a spindle-shaped and unhealthier appearance. Furthermore, although an equal number of cells was seeded, cell confluency in the cells treated with 200 µM $NiCl_2$ was similar to that of the control group, while cell confluency in the group of cells treated with 600 µM $NiCl_2$ was decreased almost by half (Figure 1).

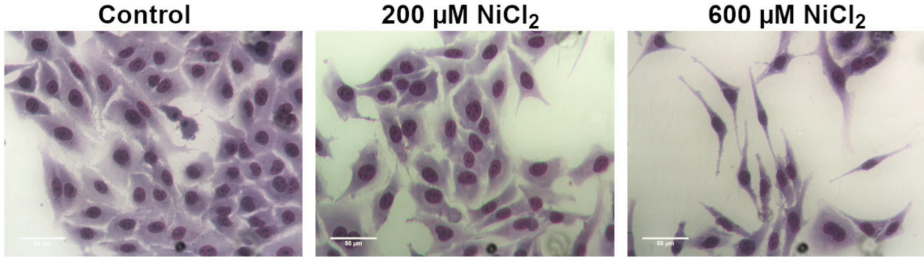

Figure 1: Effect of nickel chloride on cell morphology. Giemsa staining technique was used for determination of cellular morphological changes. All samples were examined under the same magnification level under a brightfield microscope.

$NiCl_2$: Nickel chloride

Thus, our study demonstrated that $NiCl_2$ administration changed the cellular morphology of A549 cell lines in a dose-dependent manner.

$NiCl_2$ Treatment Suppresses Cell Migration in A549 Cell Lines

For the application of $NiCl_2$ at a dose affecting cellular morphology, an *in vitro* scratch analysis was carried out to determine the changes in the migration abilities of A549 cell lines. Following the treatment of cells with 600 µM $NiCl_2$, the areas of migration were evaluated at 6, 12, 24, and 48 hours to determine migration status. The migration area in the control group was found to be significantly reduced after 12 hours when compared to the $NiCl_2$-treated group ($p<0.05$). Similarly, the cell migration was sustained at 24 and 48 hours in the control group, but was suppressed in the $NiCl_2$-treated group, and the migration area was significantly larger ($p<0.001$) (Figure 2).

The findings of the present study reveal that $NiCl_2$ treatment suppresses cell migration in A549 cell lines.

DISCUSSION

The use of metal-based compounds is of great importance in medicine. Many metals and metal-based compounds such as antimony (Sb), gold (Au), iron (Fe), silver (Ag), and platinum (Pt) are used effectively in the treatment of cancer protozoal, arthritic, malarial, and microbial diseases. Also, compounds containing a wide spectrum of metals from transition elements to main elements have been extensively studied to identify any anti-tumor activity (13). Following the years of success of the metal-based cisplatin and other platinum-based drugs on cancer treatment, great advances have been made in the use of both essential and non-essential metals and their complexes (14).

One such metal, Ni is found in vast amounts in nature and has been identified as a human carcinogen by the International Agency for Research on Cancer (6). However, no DNA damage attributable to the $NiCl_2$ Ni compound has been demonstrated, leading it to be regarded as a non-genotoxic Ni compound with weak carcinogenic effects among the other Ni compounds (8).

While various studies have demonstrated the potential carcinogenic properties of $NiCl_2$ (15-17), many others have identified its potential use for the treatment of cancer (9). Studies of several cell lines for the evaluation of the anticancer activity of $NiCl_2$ have investigated concentrations that suppress cellular proliferation by 50% (IC50) (10). The IC50 has been reported to be 1.5 mM in osteosarcoma cell lines (U2OS), 2 mM in human keratinocytes (HaCat) (10), and 400 µM in hepatocellular carcinoma cell lines (HepG2) (12), and $NiCl_2$ has been demonstrated to suppress cellular proliferation and induce apoptosis in cancer cells (10). Our study evaluated cell confluency in A549 cell lines after $NiCl_2$ treatment at doses of 200 µM and 600 µM and recorded the changes in the cellular morphology. Accordingly, the cell confluency rates did not change with a 200 µM dose, while cell confluency decreased by 50% at a dose of 600 µM. In addition, Giemsa staining revealed no significant changes in cellular morphology in the cell lines treated with 200 µM $NiCl_2$, whereas, in the groups treated with 600 µM $NiCl_2$, the cell morphology has been disrupted, with a loss of cell-to-cell contact, a decrease in the cytoplasm, and a morphological divergence from their ancestors by gaining a spindle-shaped and unhealthier appearance.

The migration of cancer cells and their ability to metastasize to distant organs leads to failure in cancer treatment and makes the greatest contribution to cancer-related deaths (18). Indeed,

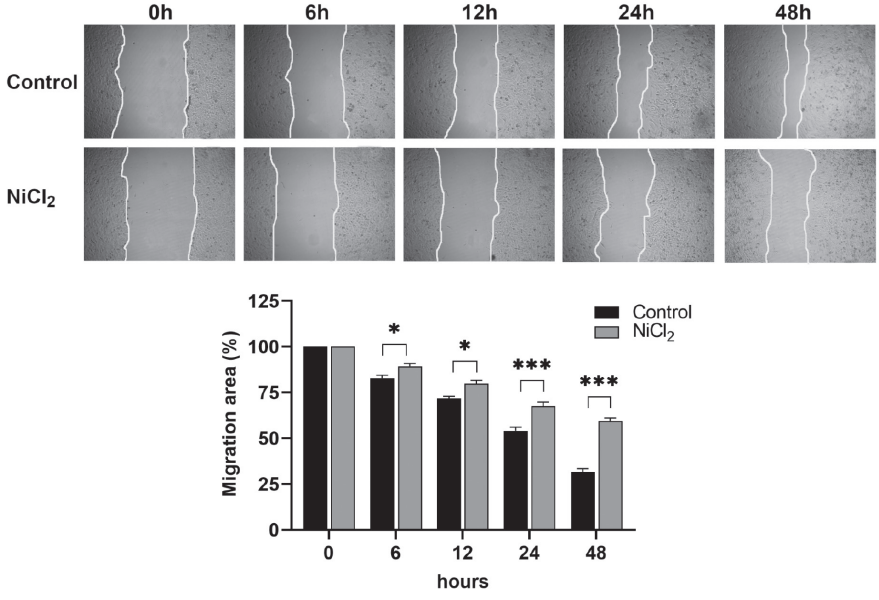

Figure 2: Effect on nickel chloride on cell migration. An *in vitro* scratch assay was used to define the migration status of A549 cells. Asterisk indicates *p<0.05, ***p<0.001

$NiCl_2$: Nickel chloride

approximately 90% of cancer-related deaths have been linked to metastasis, being a complex process involving the migration of cancer cells after separation from the primary local tumor and invasion of the surrounding tissues and colonization in distant organs (19). In the light of these data, gaining insight into the key molecular actors in the metastasis process and how to target these actors through therapeutic interventions is vital in the suppression of this process.

In a study conducted by Ota et al. (9), $NiCl_2$ was shown to decrease matrix metalloproteinase expressions significantly at messenger ribonucleic acid (mRNA) and protein levels, and to decrease the expression of angiogenic factors such as IL-8 and vascular endothelial growth factor (VEGF) at mRNA level. Moreover, the authors demonstrated that various genes involved in cancer metastasis were suppressed in mice fed with $NiCl_2$ (9). Based on these effects, $NiCl_2$ was determined to suppress cell migration and metastasis in oral squamous cell carcinoma, identifying the potential of $NiCl_2$ as a new and promising therapeutic anti-cancer agent (9). Similar to their study, our study demonstrated that $NiCl_2$ suppressed cell migration in A549 cell lines at a dose of 600 µM from 12 hours, and this effect was sustained and augmented at 24 and 48 hours after administration.

CONCLUSION

The findings of this study reveal that cell morphology was affected, and cellular migration was suppressed in non-small cell lung cancer cell lines treated with $NiCl_2$.

Ethics Committee Approval: *All assays were performed in in-vitro using commercially purchased cell lines. This study does not include any human or animal data.*

Informed Consent: *The study does not require patient consent.*

Conflict of Interest: *The authors declared no conflict of interest.*

Author Contributions: *Concept: E.G., Design: H.T.K, E.G., Data Collection or Processing: H.T.K, Ç.T., A.B., Analysis or Interpretation: H.T.K., E.G., Literature Search: H.T.K., Writing: H.T.K., Ç.T.K., A.B., E.G.*

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