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Synergistic antiproliferative effects of the cisplatin and nimotuzumab combination through modulation of EGFR/MEK/ERK signaling in neuroblastoma

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Abstract

This study investigated the synergistic cytotoxic effect of cisplatin and nimotuzumab combination in neuroblastoma cells and their impact on EGFR/MEK/ERK signaling. Human neuroblastoma cells (SH-SY5Y) were treated with cisplatin (1, 3, and 10 μM), nimotuzumab (10 μM), and their combinations (1 μM Cis + 10 μM NMTZ, 3 μM Cis + 10 μM NMTZ, and 10 μM Cis + 10 μM NMTZ) for 48 h. Cell viability was assessed using a proliferation assay, while western blot analysis measured protein expression levels. Quantitative RT-PCR was performed to determine EGFR mRNA expression. Cisplatin monotherapy reduced cell viability with survival rates of 82% (1 μM), 71% (3 μM), and 60% (10 μM) ($p<0.05$). The combination therapy resulted in enhanced cytotoxicity, with viability decreasing to 54% (1 μM Cis + 10 μM NMTZ), 44% (3 μM Cis + 10 μM NMTZ), and 25% (10 μM Cis + 10 μM NMTZ) ($p<0.05$, compared to control and single-agent treatments). Western blot data showed a significant decrease in EGFR expression in the combination groups ($p<0.05$), whereas p-EGFR levels were increased. ERK1/2 expression increased in cisplatin-treated groups but decreased with combination treatment ($p<0.01$). p-ERK1/2 levels increased in the nimotuzumab-treated groups, suggesting a compensatory activation of survival pathways. Nimotuzumab enhanced cisplatin-induced cytotoxicity in neuroblastoma cells by modulating EGFR/MEK/ERK signaling. Combination therapy reduced cell viability, downregulated EGFR expression, and disrupted ERK1/2-mediated survival mechanisms. Nimotuzumab may mitigate cisplatin resistance by preventing EGFR upregulation, offering a potential strategy to improve neuroblastoma treatment outcomes.

Keywords: Neuroblastoma, cisplatin, nimotuzumab, combination therapy

Introduction

Neuroblastoma is one of the most prevalent extracranial solid tumors, accounting for nearly 6% of all pediatric malignancies [1]. This malignancy originates from primordial neural crest cells and demonstrates highly heterogeneous biological behavior, ranging from regression in infants to aggressive metastatic disease in older children [2,3]. Despite advances in multimodal treatment approaches, including chemotherapy, radiation therapy, and immunotherapy, high-risk neuroblastoma remains a major therapeutic challenge owing to its propensity for relapse and resistance to standard therapies [4,5].

Cisplatin, a chemotherapeutic agent, is the cornerstone of neuroblastoma treatment because of its potent DNA-damaging effects. However, its clinical utility is often limited by intrinsic or acquired resistance mechanisms that involve alterations in drug uptake, DNA repair pathways, and apoptotic signaling [6,7].

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Furthermore, cisplatin-induced toxicities such as nephrotoxicity, ototoxicity, and neurotoxicity significantly affect the long-term survival of patients with malignancy [8,9]. These limitations necessitate the development of novel combination therapies to enhance cisplatin efficacy while minimizing its adverse effects.

Targeted therapy has emerged as an important strategy in cancer treatment, particularly for tumors that display aberrant receptor signaling [10]. Epidermal growth factor receptor (EGFR) is overexpressed on the membrane surface of various cancer cells, including glioblastoma and neuroblastoma, and plays an important role in proliferation, apoptosis, survival, and metastasis [11]. Inhibition of EGFR signaling has been explored as a potential therapeutic approach in neuroblastoma, as EGFR blockade can impair tumor cell growth and sensitize resistant cells to chemotherapeutic agents [3,12,13].

Clinical effectiveness in various solid tumors, including nasopharyngeal carcinoma, cervical cancer, and head and neck squamous cell carcinoma, has been shown by nimotuzumab. This humanized monoclonal antibody targets the EGFR extracellular domain [14,15]. Compared to other EGFR inhibitors, nimotuzumab exhibits a favorable safety profile because of its selective binding characteristics, reducing the incidence of severe dermatological and gastrointestinal toxicities commonly observed with other anti-EGFR agents [16,17]. Recent studies have highlighted the potential of nimotuzumab in combination with cisplatin-based chemotherapy, showing enhanced cytotoxic effects in nasopharyngeal carcinoma and cervical cancer [18,19]. However, their efficacy in neuroblastoma remains largely unexplored.

This study aimed to investigate the combined effects of nimotuzumab and cisplatin on neuroblastoma cell proliferation and on the expression of key proteins involved in the EGFR, Mitogen-Activated Protein Kinase Kinase (MEK), and Extracellular Signal-Regulated Kinase (ERK) signaling pathway. Given the role of this pathway in tumor progression and chemotherapy resistance, elucidating the molecular impact of this combination therapy may provide insights into novel treatment strategies for neuroblastoma. We hypothesized that the addition of nimotuzumab to cisplatin would enhance antiproliferative effects and modulate EGFR-dependent signaling, potentially overcoming cisplatin resistance mechanisms.

Material and Methods

Reagents

The Cell Proliferation Kit-8 was purchased from Sigma-Aldrich (St. Louis, MO, USA). RPMI-1640 and FBS (fetal bovine serum) were purchased from Biowest (Nuaillé, France). SH-SY5Y was purchased from the American Type Culture Collection (ATCC, USA). Anti-EGFR, anti-phospho-EGFR, anti-MEK1/MEK2, anti-phospho-MEK1/MEK2 (p-MEK1/MEK2), anti-ERK1/2, andanti-phospho-ERK1/2 (p-ERK1/2), were purchased from Invitrogen (Carlsbad, USA).

Cell Culture

SH-SY5Y cells were cultured in DMEM/F-12, supplemented with 10% FBS, 100 U/mL penicillin/streptomycin. The cells were cultured in a humidified incubator at 37°C in a 5% CO_2 atmosphere. The media was replaced every 48 h, and the cells were passaged upon reaching 80-90% confluency by 0.25% trypsin-EDTA.

Cell Proliferation Assay

The in vitro cytotoxicity of cisplatin, nimotuzumab, and their combination was assessed using a CCK-8 assay. SH-SY5Y cells (3×10^3 cells/well) were seeded into 96-well plates. After incubation, cells were incubated with cisplatin solutions (1, 3, and 10 μM), nimotuzumab (10 μM), or a cisplatin-nimotuzumab combination (1 μM Cis + 10 μM NMTZ, 3 μM Cis + 10 μM NMTZ and 10 μM Cis + 10 μM NMTZ) for 48 h. Then, 100 μL of 10% CCK-8 solution was added to each well and incubated for 4 h. Absorbance was measured at 450 nm using a microplate reader (Multiskan FC, Thermo Fisher Scientific, USA).

Genetic Analysis

Total RNA was meticulously isolated from SH-SY5Y neuronal cells utilizing the PureZol RNA isolation reagent (#7326890, Bio-Rad, Hercules, CA, USA). The total RNA concentration was analyzed by a NanoDrop (ND-1000, Thermo Fisher Scientific). Complementary DNA (cDNA) was synthesized using an iScript cDNA Synthesis Kit (#1708890, Bio-Rad). Analysis was performed using the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with site-specific primers, SYBR Green Master Mix (#1725270; Bio-Rad), and nuclease-free water. GAPDH was used as an internal reference gene. Primer sequences are presented in Table 1.

Table 1. Primer sequences

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
EGFR	AAAGTTAAAATTCCCGTCGCTATCAAG	TCACGTAGGCTTCATCGAGGATTTC
GAPDH	CATTGCCCTCAACGACCACTTT	GGTGGTCCAGGGGTCTTACTCC

Western Blot Analysis

Western blotting was used to assess the expression levels of Epidermal Growth Factor Receptor (EGFR) and its activated phosphorylated form (p-EGFR), as well as the downstream signaling proteins Mitogen-Activated Protein Kinase Kinases 1 and 2 (MEK1/MEK2) and their phosphorylated active forms (p-MEK1/MEK2). Additionally, the expression of Extracellular Signal-Regulated Kinases 1 and 2 (ERK1/2) and

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their phosphorylated active forms (p-ERK1/2) were evaluated following treatment with cisplatin and nimotuzumab. SH-SY5Y cells were plated and incubated overnight to ensure adherence to the treatment. Cells were then treated with varying concentrations of cisplatin (1, 3, and 10 µM), nimotuzumab (10 µM), or combination regimens (1 µM Cis + 10 µM NMTZ, 3 µM Cis + 10 µM NMTZ, and 10 µM Cis + 10 µM NMTZ) for 48 h.

Cells were digested by trypsin and lysed in RIPA buffer containing protease and phosphatase inhibitors (Santa Cruz Biotechnology, USA). Lysates were incubated on ice for 15 min. After incubation, samples were centrifuged at 20,000 × g for 20 min at 4 °C. Protein concentrations were tested by the BCA protein assay kit using a spectrophotometer. Then, samples containing equal amounts of protein (20 µg) for each group were separated according to their molecular weights using 10% SDS-PAGE. They were then transferred to nitrocellulose membranes for blotting. Blocking was performed with 5% non-fat milk for 1 h. Then membranes were incubated with specific primary antibodies at 4 °C overnight. After incubation, membranes were washed for 3 × 5 min. After a washing step, membranes were incubated with HRP-conjugated secondary antibodies at room temperature for 1 h. Protein bands were visualized using an imaging system and analyzed densitometrically (iBright Imaging Systems, Thermo Fisher Scientific).

Ethical Considerations

This study exclusively involved in vitro experiments with commercially available neuroblastoma cells (SH-SY5Y, ATCC, VA, USA), ethics committee approval was not required. All procedures complied with standard biosafety and ethical guidelines for in vitro research. Approval that ethics committee approval was not required was received from the Afyonkarahisar Health Sciences University Clinical Studies Ethics Committee (Approval Number: 2011-KAEK-2, Date: 02.07.2021).

Statistical Analysis

GraphPad Prism 8. was used to perform statistical analyses. Data were expressed as mean±standard deviation (SD). Cell viability was calculated relative to untreated controls, and group comparisons were conducted via one-way ANOVA with Tukey's post-hoc test. A p-value ≤0.05 was considered statistically significant. Gene expression data were analyzed using REST 2009 V2.0.13 software, with $p<0.05$ indicating significance.

Results

Antiproliferative Effects of Cisplatin, Nimotuzumab, and their Combinations on Neuroblastoma Cells

To assess the cytotoxic effects of cisplatin, nimotuzumab, and their combinations, SH-SY5Y neuroblastoma cells were treated with increasing concentrations of cisplatin (1, 3, and 10 µM), nimotuzumab (10 µM), and the combination regimens for 48 h. Cell viability was quantified using a CCK-8 assay.

Monotherapy with cisplatin (1, 3, and 10 µM) resulted in a dose-dependent reduction in cell viability, with survival rates of 82, 71, and 60%, respectively, compared to the control. Nimotuzumab alone (10 µM) did not exhibit significant antiproliferative effects, yielding a cell viability rate of 82%, similar to that of the 1 µM cisplatin group ($p>0.05$).

In contrast, combination therapy significantly enhanced the cytotoxic effects compared to single-agent treatments. The viability of cells treated with cisplatin + nimotuzumab combinations was 54% (1 µM Cis + 10 µM NMTZ), 44% (3 µM Cis + 10 µM NMTZ), and 25% (10 µM Cis + 10 µM NMTZ), respectively ($p<0.05$ for all vs. control and single-agent treatments) (Figure 1). These findings indicate a synergistic effect between cisplatin and nimotuzumab in the neuroblastoma cell proliferation inhibition.

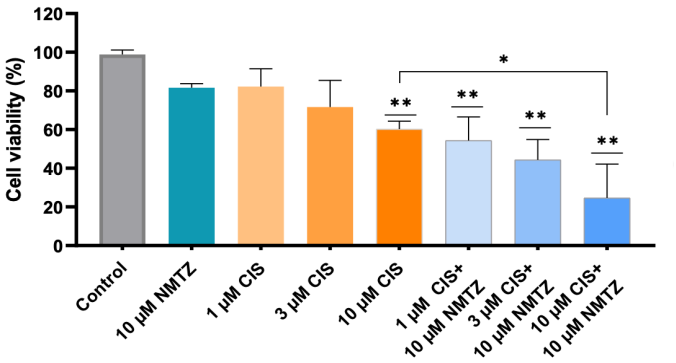

Figure 1. Antiproliferative effects of cisplatin (1, 3, 10 µM), nimotuzumab (10 µM), and their combinations in SH-SY5Y cells; Data are shown as mean±SD (n=3); $p<0.05$ and *$p<0.001$ indicate statistical significance

Effects of Cisplatin and Nimotuzumab on EGFR/MEK/ERK Protein Expression

The expression levels of EGFR, phosphorylated EGFR (p-EGFR), MEK1/MEK2, phosphorylated MEK1/MEK2 (p-MEK1/MEK2), ERK1/2, and phosphorylated ERK1/2 (p-ERK1/2) were analyzed via western blot following treatment with cisplatin, nimotuzumab, and their combinations.

EGFR and p-EGFR Expression

Cisplatin treatment (1, 3, and 10 µM) alone did not induce significant changes in the total EGFR expression ($p>0.05$). However, p-EGFR levels were significantly elevated in the 10 µM nimotuzumab group compared with the control ($p<0.05$), suggesting the activation of EGFR phosphorylation. Notably, combination treatments (3 µM Cis + 10 µM NMTZ and 10 µM Cis + 10 µM NMTZ) resulted in a marked decrease in total EGFR expression, while p-EGFR levels increased, indicating pathway modulation upon dual treatment.

MEK1/MEK2 and p-MEK1/MEK2 Expression

MEK1/MEK2 expression remained unchanged across the cisplatin and nimotuzumab monotherapy groups ($p>0.05$). However, combination treatment (3 µM Cis + 10 µM NMTZ and

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10 μM Cis + 10 μM NMTZ) led to a significant reduction in total MEK1/MEK2 levels compared with the control group ($p<0.05$). Phosphorylated MEK1/MEK2 levels did not exhibit significant alterations across the treatment groups ($p>0.05$).

ERK1/2 and p-ERK1/2 Expression

Treatment with cisplatin alone (1, 3, and 10 μM) led to an increase in ERK1/2 expression compared with that in the control ($p<0.05$). However, in the 10 μM Cis + 10 μM NMTZ group, ERK1/2 expression was significantly reduced ($p<0.01$), suggesting an inhibitory effect of the combination therapy on this signaling axis. Notably, p-ERK1/2 expression increased in a dose-dependent manner following nimotuzumab treatment, with the overexpression observed in the combination groups (Figure 2).

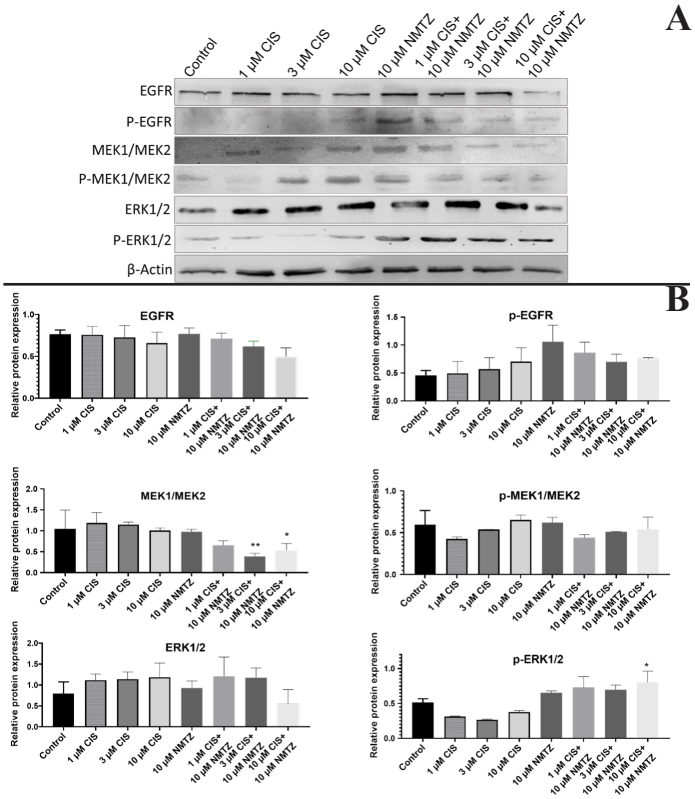

Figure 2. A. Western blot analysis of EGFR, p-EGFR, ERK1/2, p-ERK1/2, MEK1/MEK2, and p-MEK1/MEK2 expression following 48-hour treatment with cisplatin and/or nimotuzumab; **B.** Densitometric analysis of protein expression (n=3); $p<0.05$; *$p<0.01$ vs. control

EGFR Gene Expression Analysis by qRT-PCR

To further investigate the effect of treatment on EGFR signaling, EGFR mRNA expression levels were quantified using real-time PCR. EGFR mRNA expression was significantly upregulated in the 1 μM Cis and 3 μM Cis + 10 μM NMTZ groups (*fold-change: 1.54, $p<0.05$, and 1.46, $p<0.001$, respectively). In contrast, EGFR mRNA expression was significantly downregulated in the 10 μM Cis and 10 μM NMTZ groups (*fold-change: 0.66, $p<0.001$; 0.27, $p<0.001$, respectively). No significant alterations in gene expression were observed in the 3 μM Cis, 1 μM Cis + 10 μM NMTZ, or 10 μM Cis + 10 μM NMTZ groups ($p>0.05$). The findings showed dose-dependent modulation of EGFR transcription upon cisplatin and nimotuzumab exposure (Figure 3).

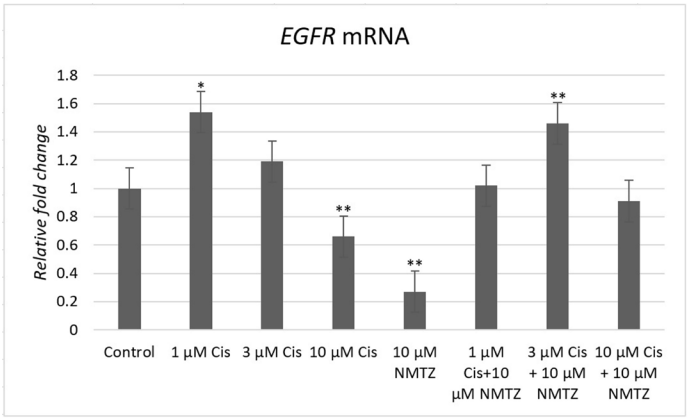

Figure 3. The mRNA expression of EGFR in SH-SY5Y neuroblastoma cells treatment with cisplatin, nimotuzumab, and their combinations. The column graph is shown as fold change relative to the control group. $p<0.05$; *$p<0.001$; GAPDH is a reference gene for normalization

Discussion

The Antiproliferative Effects of Cisplatin and Nimotuzumab

The findings of this study demonstrated that the combination of cisplatin and nimotuzumab significantly increased the antiproliferative effects in SH-SY5Y neuroblastoma cells compared to monotherapy. Although cisplatin exhibited a cytotoxic effect, nimotuzumab alone did not significantly alter cell viability. This aligns with previous studies indicating that anti-EGFR monoclonal antibodies primarily function by inhibiting downstream signaling pathways rather than by directly inducing cytotoxicity [20-23]. However, when combined with cisplatin, nimotuzumab substantially potentiated the growth inhibition, suggesting a synergistic interaction. This effect is likely mediated by enhanced inhibition of the EGFR/MEK/ERK signaling pathway, which plays a critical role in neuroblastoma proliferation and survival. The synergy between cisplatin and nimotuzumab is particularly relevant for overcoming chemotherapy resistance, as previous studies have highlighted the compensatory upregulation of survival pathways in response to cisplatin treatment [22,23]. Li et al. demonstrated that targeting multiple signaling pathways in cisplatin-resistant neuroblastoma cells improved therapeutic efficacy, supporting the potential clinical relevance of our findings [24]. Similarly, Michaelis et al. reported that inhibition of EGFR sensitized neuroblastoma cells to chemotherapeutic agents, reinforcing the hypothesis that EGFR-targeted therapies can enhance cisplatin responsiveness [23].

The lack of rebound effect after prolonged administration of nimotuzumab in patients with poor-prognosis central nervous system tumors suggests that this treatment is safe and feasible as a new therapeutic option [24]. Nimotuzumab has demonstrated clinical benefits, including increased survival and improved

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functional status in patients with malignant brain tumors [25]. Notably, nimotuzumab has increased survival rates in patients with high-grade glioma when used alongside radio-chemotherapy [26].

Moreover, its favorable safety profile and targeted mechanism of action make nimotuzumab a promising candidate for integration into multimodal treatment strategies for aggressive CNS tumors [27].

EGFR Signaling Modulation and Cisplatin Sensitization

EGFR is a key regulator of tumor progression, proliferation, and resistance in neuroblastoma. Our results indicated that cisplatin alone did not significantly alter total EGFR expression, whereas nimotuzumab treatment decreased EGFR protein levels. Notably, the combination treatment resulted in a more pronounced reduction in EGFR expression, suggesting that nimotuzumab counteracted cisplatin-induced compensatory upregulation of EGFR. The results of our study were consistent with previous studies reporting that neuroblastoma cells show increased EGFR expression as an adaptive response to cisplatin exposure [21,23,28]. Roessler et al. observed that cisplatin-resistant neuroblastoma cells exhibit higher EGFR levels, indicating a mechanism by which tumor cells evade cisplatin-induced cytotoxicity [29]. Our data suggested that nimotuzumab mitigates this resistance mechanism by effectively suppressing EGFR activation, thereby restoring cisplatin sensitivity. Additionally, real-time PCR analysis showed a dose-dependent modulation of EGFR mRNA levels, with a significant downregulation observed in the high-dose combination groups. This suggests that nimotuzumab exerts its effects on EGFR not only at the protein level but also at the transcriptional level, contributing to the enhanced therapeutic efficacy observed in our study.

MEK/ERK Pathway Inhibition and Tumor Suppression

The MEK/ERK signaling cascade is a crucial downstream pathway of EGFR that regulates tumor cell proliferation. Our data demonstrated that total MEK1/MEK2 expression levels were significantly reduced in the combination therapy groups, whereas phosphorylation levels remained relatively unchanged. In contrast, ERK1/2 expression was elevated in cisplatin-treated cells but significantly downregulated in the nimotuzumab combination group, indicating a potential mechanism by which dual therapy inhibits tumor growth. The observed increase in phosphorylated ERK1/2 levels suggests activation of compensatory survival mechanisms, a phenomenon commonly reported in response to EGFR-targeted therapies. Schnidar et al. previously described a similar compensatory loop in oncogenic transformation models, where EGFR inhibition led to feedback activation of ERK signaling, thereby sustaining cell survival [30]. Studies have further demonstrated that increased phosphorylated ERK expression correlates with tumor aggressiveness, reinforcing the clinical importance of targeting this pathway in neuroblastoma [31,32]. Despite the upregulation of phosphorylated ERK1/2, the significant downregulation of total ERK1/2 levels in the combination groups suggested that cisplatin-nimotuzumab therapy effectively disrupted the oncogenic signaling cascade, contributing to the observed antiproliferative effects.

EGFR-targeted therapies have been extensively explored in neuroblastoma and other malignancies, yet their clinical efficacy has been limited by resistance mechanisms and toxicity concerns [20,23]. Nimotuzumab is an EGFR inhibitor because of its selective binding properties, which reduce off-target effects while maintaining its anti-tumor activity. Previous studies have shown that cetuximab, another anti-EGFR, has shown promising results in head and neck cancers when combined with cisplatin but exhibited limited efficacy in neuroblastoma models [21,23]. Gefitinib, an EGFR tyrosine kinase inhibitor, has also been investigated in neuroblastoma; however, its clinical application has been hindered by acquired resistance mechanisms and incomplete pathway inhibition [3,33]. The findings of our study suggest that nimotuzumab, when used in combination with cisplatin, effectively enhances therapeutic efficacy by modulating EGFR signaling without inducing excessive compensatory activation. This contrasts with gefitinib, which has been reported to result in paradoxical pathway activation in resistant neuroblastoma cells [21,23]. Compared to other EGFR-targeted strategies, the dual action of nimotuzumab, which blocks ligand binding while allowing partial receptor function, may contribute to its unique ability to potentiate cisplatin-induced cytotoxicity while minimizing the development of resistance.

This study presents the therapeutic potential of cisplatin-nimotuzumab combination therapy for neuroblastoma. Given the observed synergy between these agents, future investigations should focus on elucidating the additional mechanisms of resistance and optimizing combination regimens. One potential avenue for further research is the role of alternative compensatory pathways, such as the PI3K/AKT axis, which has been implicated in neuroblastoma survival and therapy resistance. Additionally, evaluating the efficacy of this combination therapy in cisplatin-resistant neuroblastoma models would provide valuable insight into its clinical applicability. Another promising approach is the co-administration of MEK inhibitors, as dual targeting of EGFR and MEK signaling has shown enhanced therapeutic effects in preclinical models. The observed increase in phosphorylated ERK1/2 levels in our study suggests that combining nimotuzumab and cisplatin with an MEK inhibitor may further enhance tumor suppression by overcoming feedback activation mechanisms.

In the presented study, it was shown that nimotuzumab contributes to the anti-mitotic effect of cisplatin in neuroblastoma cells by regulating the EGFR/MEK/ERK signaling pathway. These findings are consistent with those of previous reports that emphasized the role of EGFR in neuroblastoma progression and drug resistance. However, the observed upregulation of phosphorylated ERK1/2 suggests activation of compensatory survival pathways, indicating that additional therapeutic interventions may be necessary to fully exploit the benefits of EGFR inhibition. The promising results presented here highlight the potential of nimotuzumab as a valuable adjunct to cisplatin

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therapy in neuroblastoma, warranting further preclinical and clinical investigations to validate its efficacy and optimize the treatment strategies.

Study Limitations

The study showed that the combination of cisplatin and nimotuzumab increased the antiproliferative effect on neuroblastoma cells, but there are still some limitations. First, the study was conducted in vitro using the SH-SY5Y neuroblastoma cell line, which may not fully represent the complexity of neuroblastoma tumors in a clinical setting. Tumor microenvironmental factors, such as immune cell interactions, extracellular matrix components, and hypoxic conditions, can influence the efficacy of cisplatin-nimotuzumab combination therapy; however, these aspects were not addressed in this model.

While the study demonstrated significant alterations in EGFR/MEK/ERK signaling, other potential compensatory pathways, such as PI3K/AKT/mTOR, were not evaluated. Activation of alternative survival pathways in response to EGFR inhibition is a well-documented resistance mechanism, and its role in neuroblastoma should be further investigated to determine whether additional pathway inhibitors could enhance the therapeutic efficacy of this combination.

Future studies employing preclinical studies would be instrumental in confirming the translational potential of the findings and evaluating the pharmacokinetic and toxicity profiles to assess the clinical feasibility of this combination therapy.

Conclusion

This study demonstrated that the combination of cisplatin and nimotuzumab significantly enhanced the antiproliferative effects on SH-SY5Y neuroblastoma cells compared with monotherapy. This synergy is mediated through modulation of the EGFR/MEK/ERK signaling pathway, with combination treatment reducing EGFR expression, suppressing MEK/ERK activation, and enhancing cell death. These findings suggest that nimotuzumab mitigates compensatory EGFR upregulation in response to cisplatin, enhances chemosensitivity, and overcomes a key resistance mechanism.

Real-time PCR and Western blot analyses revealed that cisplatin-induced ERK1/2 activation was attenuated by nimotuzumab, supporting the hypothesis that dual inhibition of the EGFR and signaling pathways may provide a more effective therapeutic approach. The selective action of nimotuzumab, which targets tumor-associated EGFR while minimizing off-target effects, presents a potential advantage over other EGFR inhibitors with dose-limiting toxicities.

These findings show the therapeutic effect of combining cisplatin with nimotuzumab for neuroblastoma treatment, offering a promising strategy to enhance cisplatin efficacy, reduce resistance, and improve clinical outcomes. Further preclinical and clinical investigations are warranted to validate these results in vivo models and patient cohorts, assess potential resistance mechanisms, and determine the optimal dosing strategies for clinical translation.

Conflict of Interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

This study was supported by the Afyonkarahisar Health Sciences University, Scientific Research Projects Commission (Grant Number: 21.GENEL.017).

Ethical Approval

Not necessary for this manuscript. This study utilized a commercially available neuroblastoma cell line; therefore, ethics committee approval was not required. Approval that ethics committee approval was not required was received from the Afyonkarahisar Health Sciences University Clinical Studies Ethics Committee.. (Approval Number: 2011-KAEK-2, Date: 02.07.2021).

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