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Original Article

A Synergistic Approach: Enhanced Apoptotic and Proliferative Inhibition of Cisplatin by Nanocurcumin in HeLa Cells

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Abstract: Cisplatin is a widely used chemotherapeutic agent for cervical cancer, but its effectiveness is often limited by cellular resistance and severe side effects. Curcumin has demonstrated potential to enhance cisplatin's anticancer effects by inducing apoptosis and inhibiting cell proliferation; however, its clinical use is constrained by poor solubility and low bioavailability. To overcome these limitations, liposomal nanocurcumin was developed. This study aimed to evaluate the synergistic effects of cisplatin and nanocurcumin on Bax and BrdU expression as markers of apoptosis and proliferation in HeLa cervical cancer cells. A true experimental post-test only design was employed with five groups: HeLa cell control (no treatment), positive control (cisplatin 5 μg/mL), and three treatment groups receiving cisplatin 2.5 μg/mL combined with nanocurcumin at 25, 50, and 100 μg/mL. Cells were incubated for 48 hours and analyzed using flow cytometry. The combination treatment significantly increased Bax expression and reduced BrdU expression compared to cisplatin alone, with the strongest effect observed at 100 μg/mL nanocurcumin. These findings suggest that nanocurcumin may serve as a promising adjuvant to enhance the therapeutic efficacy of cisplatin by promoting apoptosis and suppressing proliferation in cervical cancer cells.

Keywords: Cervical cancer; Cisplatin; Nanocurcumin; Bax; BrdU

1. INTRODUCTION

Curcumin, the main active compound from the rhizome of Curcuma longa L., has long been recognized for its various pharmacological benefits, including its anticancer properties [1]. The anticancer mechanisms of curcumin include the selective increase of reactive oxygen species (ROS) in cancer cells, regulation of apoptosis-related proteins such as Bax, and inhibition of cell proliferation pathways [2]. However, one of the major challenges in utilizing curcumin is its limited solubility and bioavailability, which reduces its therapeutic effectiveness in its natural form. To address this, liposomal nanocurcumin formulations have been developed, which encapsulate curcumin in lipid vesicles to enhance stability, absorption, and therapeutic effectiveness in cancer cells [3].

In the context of cervical cancer therapy, nanocurcumin can serve as an adjunct to conventional therapies such as cisplatin, a platinum-based chemotherapy. Cisplatin works by damaging the DNA of cancer cells, inducing apoptosis, and inhibiting cell proliferation. Despite its effectiveness, the use of cisplatin is often limited by cancer cell resistance and significant toxic side effects [4,5]. Therefore, the combination of cisplatin with curcumin offers a synergistic approach that could potentially enhance the apoptotic response and simultaneously suppress cell proliferation [6].

One of the key pathways in anticancer effects is the induction of apoptosis through the intrinsic mitochondrial pathway, which is heavily influenced by the expression of pro-apoptotic proteins such as Bax [7]. On the other hand, cancer cells often exhibit increased proliferation, marked by high levels of Bromodeoxyuridine (BrdU), a marker for DNA synthesis during the S phase of the cell cycle [8]. Low Bax expression and high BrdU expression reflect the cancer cell's ability to survive and proliferate aggressively. Therefore, therapeutic approaches that can increase Bax expression and decrease BrdU expression become an ideal strategy to suppress cervical cancer development.

Based on this background, the combination approach of nanocurcumin and cisplatin is expected to provide a stronger effect in triggering cancer cell death and inhibiting proliferation. This study aims to evaluate the impact of the combination of nanocurcumin and cisplatin on the expression of Bax as an indicator of apoptosis and BrdU as an indicator of proliferation in HeLa cervical cancer cell cultures. The findings of this study are expected to provide scientific evidence for the use of natural-based nanotechnology as a potential adjuvant in enhancing the effectiveness of cervical cancer therapy.

2. MATERIALS AND METHODS

This true experimental in vitro study, with a post-test only design, was conducted using HeLa cell cultures treated with a combination of nanocurcumin at various concentrations and cisplatin, followed by evaluation of Bax and BrdU expression. The cisplatin used in this study was a pharmaceutical-grade product from Kalbe, while the nanocurcumin was obtained from the Pharmaceutical Laboratory, Faculty of Medicine, Universitas Brawijaya. This research complied with ethical research guidelines and received approval from the Health Research Ethics Committee, Faculty of Medicine, Universitas Brawijaya, under approval numbers 488/EC/KEPK-S2/12/2024 for the analysis of BrdU expression and 02/EC/KEPK-S2/01/2024 for the analysis of Bax expression.

2.1. Nanoliposomal Synthesis

Curcuma rhizomes were extracted using 96% ethanol via Soxhlet extraction at 60°C for 8 hours, followed by purification through hexane extraction, drying, and crystallization with isopropyl alcohol. Curcumin-loaded nanoliposomes were synthesized using the thin-film hydration method, in which curcumin, soy phosphatidylcholine, cholesterol, and Tween-80 were dissolved in chloroform, followed by drying and hydration with PBS-Tween-80. Particle size analysis using a Particle Size Analyzer (PSA, Shimadzu SALD-7500nano) revealed an average size of 32 nm, within the nanoliposomal range (20–150 nm), supporting stability, absorption, and delivery efficiency. High-Performance Liquid Chromatography (HPLC) analysis confirmed the purity and stability of the nanocurcumin.

2.2. Cell Culture and Treatment

HeLa cells were thawed at 37°C and cultured in complete medium consisting of RPMI-1640 (Gibco), 10% FBS (Biowest), and 1% penicillin-streptomycin (Gibco). The cells were incubated at 37°C with 5% CO_2, with medium changes every 1–2 days. Once the cells reached 70–80% confluence, they were harvested using Trypsin-EDTA (Gibco) and seeded at a density of 5×10^5 cells per well in a 6-well plate (SPL), followed by 24 hours of incubation. The cells were divided into five groups: a HeLa cell control group (medium only), a positive control group (HeLa cells treated with cisplatin 5 μg/mL), and three treatment groups receiving combinations of cisplatin (2.5 μg/mL) with nanocurcumin at concentrations of 25, 50, and 100 μg/mL. After 48 hours of treatment, all cells were harvested for protein expression analysis using flow cytometry.

2.3. Flow Cytometry Analysis

The harvested cells were fixed using Nuclear Factor Fixation Buffer (for BrdU) and Cyto-Fast™ Fix/Perm Buffer (for Bax). Permeabilization was performed with Nuclear Factor Permeabilization Buffer and Cyto-Fast™ Perm Wash Solution. Cells were incubated with PE-conjugated Bax Polyclonal Antibody (bs-0127M-PE, Bioss) for 20 minutes for detection, and PercP conjugated BrdU Polyclonal Antibody (bs-0489R-PerCP, Bioss) in the dark at room temperature for 30 minutes. After staining, the cells were rinsed, suspended in 300 μL of buffer, and analyzed using a BD FACSCalibur flow cytometer. Data were processed using CellQuest Pro software to quantify fluorescence intensity, reflecting the expression of Bax and BrdU.

2.4. Statistical Analysis

Data analysis was performed using IBM SPSS Statistics software version 26. A one-way ANOVA was conducted, followed by Tukey's HSD post-hoc test for further analysis. A p-value of < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Effect of Combination Therapy on Bax Expression

The results of this study indicate that a 5 μg/mL concentration of cisplatin significantly increases Bax expression compared to the HeLa cell control group, while the combination of 2.5 μg/mL cisplatin with nanocurcumin shows a progressively greater increase in expression as the nanocurcumin concentration increases, with the highest expression achieved at a concentration of 100 μg/mL. Differences in Bax expression among groups can be seen in Figure 1. An increase in Bax protein expression reflects the activation of the intrinsic apoptosis pathway, which is crucial in the cell's response to chemotherapy [7].

To better understand the cause of the increased Bax expression, it is important to consider the possible involvement of molecular pathways in the cell's response to this combination treatment. One key mechanism likely contributing to this is the increased production of ROS. Zhang et al. (2023) found that curcumin increases ROS in HeLa cells and triggers apoptosis through Bax activation and the caspase cascade [9]. High levels of ROS can lead to mitochondrial stress, triggering Bax upregulation and initiating the intrinsic apoptosis pathway. Furthermore, the activation of p53 protein is also believed to play a role in enhancing Bax expression. P53 acts as a transcription factor that induces the expression of proapoptotic genes, including Bax, in response to

cellular stress and DNA damage. Previous studies have demostrated that curcumin activates p53, leading to increased Bax expression, thereby supporting its role in apoptosis induction [10; 11].

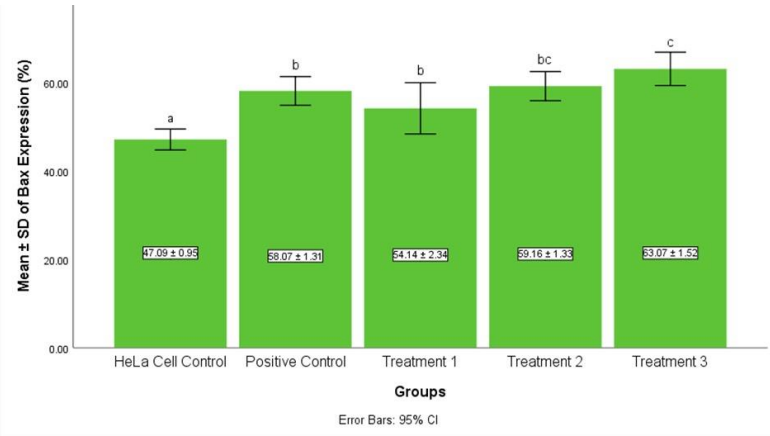

Figure 1. Different superscript letters indicate a significant difference ($p < 0.05$), while the same superscript letters indicate no significant difference ($p > 0.05$)

Overall, these findings suggest that the combination of nanocurcumin and cisplatin can trigger apoptosis through increased ROS production and p53 activation, ultimately enhancing Bax expression. This strengthens the hypothesis of a synergistic effect of the combination in activating the apoptosis pathway, although further studies are needed to investigate the involvement of other apoptosis molecules and their relevance in clinical therapy.

3.2. Effect of Combination Therapy on BrdU Expression

In this study, 5 μg/mL cisplatin significantly reduced BrdU expression compared to the HeLa cell control, while the combination of 2.5 μg/mL cisplatin with nanocurcumin showed a stronger reduction in BrdU expression as the nanocurcumin concentration increased, with the greatest effect observed in the 100 μg/mL nanocurcumin combination group. The differences in BrdU expression among the groups are illustrated in Figure 2. A decrease in BrdU expression reflects the effectiveness of chemotherapeutic agents in inhibiting cell proliferation by disrupting DNA synthesis during the S phase of the cell cycle [8].

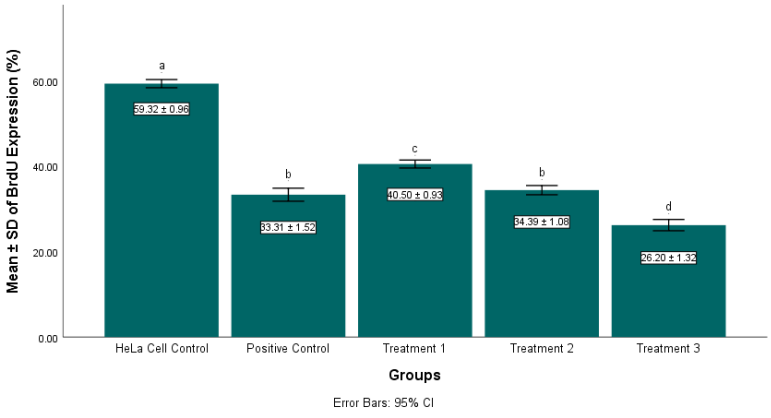

Figure 2. Different superscript letters indicate a significant difference ($p < 0.05$), while the same superscript letters indicate no significant difference ($p > 0.05$)

These results are consistent with studies in BeWo cell cultures showing that the combination of nanocurcumin and methotrexate significantly reduced BrdU expression, with the highest effect observed at a concentration of 200 μg/mL nanocurcumin. On a molecular level, nanocurcumin is known to disrupt the function of Cyclin D1 and Cyclin E1 complexes, thereby inhibiting the G1 to S phase transition, which is crucial in controlling cell proliferation [12].

These findings suggest that the combination of cisplatin and nanocurcumin may be an effective strategy to suppress cervical cancer cell proliferation. However, the specific mechanism of BrdU in the context of this combination therapy remains not fully understood, and further exploration is needed to better elucidate the cell proliferation pathways involved.

4. CONCLUSION

This study provides important insights into the potential of nanocurcumin as an adjuvant therapy in the treatment of cervical cancer, particularly in enhancing the efficacy of cisplatin. The findings demonstrate that the combination of 100 μg/mL nanocurcumin and 2.5 μg/mL cisplatin (Treatment 3) resulted in the most optimal outcomes, with a significant increase in Bax expression (63.07 ± 1.52) and the greatest reduction in BrdU expression (26.20 ± 1.32) in HeLa cells. These results highlight nanocurcumin's ability to enhance apoptosis through Bax upregulation and suppress proliferation via BrdU downregulation. Therefore, the results of this study are relevant as a reference in the development of cancer therapies, with a focus on improving therapeutic efficacy.

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Conflicts of interest: The authors declare no conflict of interest.

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