Korean Journal of
CLINICAL LABORATORY SCIENCE

ORIGINAL ARTICLE

Synergistic Inhibition of Burkitt's Lymphoma with Combined Ibrutinib and Lapatinib Treatment

Chae-Eun YANG, Se Been KIM, Yurim JEONG, Jung-Yeon LIM

Department of Biomedical Laboratory Science, Inje University, Gimhae, Korea

Ibrutinib과 Lapatinib 병용 치료에 의한 버킷림프종의 상호 작용적 억제

양채은, 김세빈, 정유림, 임정연

인제대학교 임상병리학과

ARTICLE INFO

ABSTRACT

Burkitt's lymphoma is a distinct subtype of non-Hodgkin's lymphoma originating from B-cells that is notorious for its aggressive growth and association with immune system impairments, potentially resulting in rapid and fatal outcomes if not addressed promptly. Optimizing the use of Food and Drug Administration-approved medications, such as combining known safe drugs, can lead to time and cost savings. This method holds promise in accelerating the progress of novel treatments, ultimately facilitating swifter access for patients. This study explores the potential of a dual-targeted thera-peutic strategy, combining the bruton tyrosine kinase-targeting drug Ibrutinib and the epidermal growth factor receptor/human epidermal growth factor receptor-2-targeting drug Lapatinib. Ramos and Daudi cell lines, well-established models of Burkitt's lymphoma, were used to examine the impact of this combination therapy. The combination of Ibrutinib and Lapatinib inhibited cell proli-feration more than using each drug individually. A combination treatment induced apoptosis and caused cell cycle arrest at the S and G2/M phases. This approach is multifaceted in its benefits. It enhances the efficiency of the drug development timeline and maximizes the utility of currently available resources, ensuring a more streamlined and resource-effective research process.

INTRODUCTION

Burkitt's lymphoma primarily arises from B-cells. It is notorious for its rapid growth, often leading to life-threatening conditions when left untreated [1]. This malignancy predominantly affects children and young adults, necessitating a better understanding of its epi-demiology, causative factors, and available treatment options [2]. Burkitt's lymphoma is relatively rare, but its severity demands effective therapeutic approaches. While its exact etiology remains under investigation, certain contributing factors such as immunosuppression and Epstein-Barr virus infection have been explored. Understanding the incidence, treatment modalities, and potential risk factors for Burkitt lymphoma is pivotal in the pursuit of improved patient outcomes [3].

Ibrutinib, a powerful tyrosine kinase inhibitor, has arisen as a promising treatment option for diverse B-cell

Corresponding author: Jung-Yeon LIM
Department of Biomedical Laboratory Science, Inje University, 197 Inje-ro, Gimhae 50834, Korea
E-mail: limjy@inje.ac.kr
ORCID: https://orcid.org/0000-0001-5903-8810

malignancies, such as chronic lymphocytic leukemia and mantle cell lymphoma [4]. Its mechanism of action involves blocking the B-cell receptor signaling pathway, which is a pivotal element in the survival and growth of B-cell lymphoma. Given its demonstrated efficacy and relatively manageable side effect profile, Ibrutinib has generated substantial interest in the context of lymphoma therapy [5].

Conversely, Lapatinib is a dual-tyrosine kinase inhibitor primarily developed for the treatment of human epidermal growth factor receptor 2 (HER2)-positive breast cancer. Lapatinib has shown remarkable success in arresting the growth of tumors driven by HER2 overexpression [6]. Its mechanism of action extends to inhibiting the intracellular signaling pathways responsible for cancer cell proliferation. While Lapatinib has primarily been utilized in breast cancer, its potential in addressing other malignancies is an intriguing avenue of research [7].

Epidermal growth factor receptor (EGFR)/HER2 signaling pathway activates the RAS-RAF-MEK-ERK cascade to a higher stage of c-MYC. Bruton tyrosine kinase (BTK) is a B cell receptor signaling mediator involved in inducing MYC. Therefore, to counteract overexpression of pathways that may lead to tumorigenesis, we sought to see a combination of drug combinations of BTK inhibitor and EGFR/HER2 inhibitor [8].

Despite their distinct primary applications, Ibrutinib and Lapatinib share a commonality in their ability to influence intracellular signaling pathways important for the survival and growth of cancer cells. The combination of these two agents offers the potential for a synergistic effect, where simultaneous targeting of multiple pathways may enhance the overall therapeutic response. In diseases like Burkitt lymphoma, where aggressive treatment is imperative, this combination therapy holds promise. In this study, we goal to investigate the individual and combined effects of Ibrutinib and Lapatinib, seeking to elucidate the potential benefits of this approach in the context of Burkitt lymphoma, considering both the cellular characteristics and underlying mechanisms of this malignancy.

MATERIALS AND METHODS

1. Cell Lines and Cell Culture

In this study, Burkitt's lymphoma cell lines including, Ramos and Daudi cells were used. These cell lines were obtained from Korean Cell Line Bank. These cell lines were grown in RPMI 1640 medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco), 2 mM L-glutamine (Gibco), 1% antibiotics (10 U/mL penicillin and 10 g/mL streptomycin; Gibco). All cell lines were incubated at 37℃ and 5% CO_2.

2. Drug Preparations

Ibrutinib (BTK inhibitor) and Lapatinib (HER2/neu-EGFR inhibitor) were purchased from MedChemExpress. Both were dissolved in dimethyl sulfoxide as recommended by the manufacturer and stored at −80℃. All these drugs were diluted with the RPMI 1640 medium with 10% heat-inactivated FBS, 2 mM L-glutamine, and 1% antibiotics before being used for the treatment of cell lines.

3. Cell Proliferation Assay

Cell growth was assessed using the CCK-8 (Dojindo Laboratories Co., Ltd.) assay according to the manufacturer's protocol. Ramos and Daudi cell lines were seeded at an initial cell density of 2×10^4 cells/100 μL culture medium in 96-well plates. These cells were treated with various doses of Ibrutinib and Lapatinib drugs without any drugs, 2.5 μM, 5 μM, and 10 μM for each drug. The synergistic effects were assessed using 5 μM for both drugs. Cultures were maintained at 37℃ in a 5% CO_2 atmosphere. CCK-8 solution was added in increments of 10 μL to each well after 24 hours, 48 hours, and 72 hours. The plates were incubated for 1~4 hours in a CO_2 incubator and the optical density was measured at 450 nm using a microplate reader.

4. RNA Extraction and cDNA Synthesis

The 5×10^5 cells treated with or without drugs same as described. Total RNA was extracted using TRIzol

reagent (Invitrogen) and the concentration of RNA was measured using the NanoDrop (Thermo Fisher Scientific). These were reverse transcribed using High Capacity RNA-to-cDNA Kit (Applied Biosystems) following to the manufacturer's protocol. The cDNA was synthesized using Thermocycler (Bio-Rad Laboratories, Inc.) with cycling conditions used include primer annealing at 25℃ for 10 minutes, DNA polymerization at 37℃ for 120 minutes and finally reverse transcriptase deactivation at 85℃ for 5 minutes. The synthesized cDNA was stored at −20℃ before further use.

5. Real-time Reverse Transcription Polymerase Chain Reaction

Real-time reverse transcription polymerase chain reaction (RT-PCR) was performed using the TB Green® Fast qPCR Mix (TaKaRa Bio Inc.) using the manufacturer's protocols: Hold 1 cycle for 30 seconds at 95℃, 2 step PCR 40 cycles for 5 seconds at 95℃ and 30 seconds at 60℃. Add the dissociation steps consisting of 15 seconds at 95℃, 30 seconds at 60℃ and 15 seconds at 95℃ for 1 cycle. For detection the mechanisms of the treated drugs in Burkitt's lymphoma, the following gene-specific primers were used: *p53* (forward: 5'-GTTCCGAG AGCTGAATGAGG-3'; reverse: 5'-TCTGAGTCAGGCCCTT CTGT-3'), *Bax* (forward: 5'-CTGCAGAGGATGATTGCCG-3'; reverse: 5'-TGCCACTCGGAAAAAGACCT-3'), and *GAPDH* (forward: *5'-CCACTCCTCCACCTTTGACG-3'*; reverse: *5'-CCACCACCCTGTTGCTGTAG-3*). For quantification, relative mRNA expression of specific genes was calculated using the $2^{-\Delta\Delta Ct}$ method, after normalization to *GAPDH* expression.

6. Cell Cycle Analysis

The 5×10^5 cells were treated with the drugs described. After incubating for 72 hours in CO_2 incubator, the cells were harvested and centrifuged 2,000 rpm 5 minutes with DPBS (Gibco). These cells were performed twice for wash and discarded the supernatant. For cell cycle analysis, the Annexin V-PI Apoptosis Kit (BioVision Inc.) were used following the recommended methods. The cell cycle was detected using the BD LSRFortessa (BD Biosciences).

7. Statistical Analysis

Each experiment was repeated at least three times to ensure the reproducibility of the results. Statistical significance was determined using Student's two-tailed t-test and one-way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. Statistical analysis was performed in GraphPad Prism software. Differences between expression levels at a given time point were evaluated by χ^2 contingency analysis. In all analyses, *P*-values less than 0.05 were considered to indicate statistical significance.

RESULTS

1. Ibrutinib (BTK Inhibitor) and Lapatinib (EGFR/HER2 Inhibitor) Inhibit Cell Growth in Burkitt's Lymphoma Cells

We employed a cell proliferation assay to investigate the impact of Ibrutinib and Lapatinib treatments on the growth of Burkitt's lymphoma cells. Both Ramos and Daudi cell lines were subjected to varying concentrations of Ibrutinib and Lapatinib (ranging from 2.5 μM to 10 μM) for different time intervals (24, 48, and 72 hours) (Figure 1). The results of our experiments conclusively revealed that the treatment with Ibrutinib and Lapatinib led to a marked inhibition of Burkitt's lymphoma cell proliferation.

2. Ibrutinib and Lapatinib Significantly Inhibit Cell Proliferation in MS1943 Burkitt's Lymphoma Cells

In order to assess the inhibitory effects of Ibrutinib and Lapatinib on Burkitt's lymphoma cell proliferation in an in vitro setting, we conducted cell proliferation assay analyses on cultured cells, both with and without the presence of these two drugs. Our findings revealed that the combination of Ibrutinib and Lapatinib led to a substantial reduction in Ramos and Daudi cell growth when compared to the individual drug treatments, particularly at the 72-hour time point (Figure 2). These
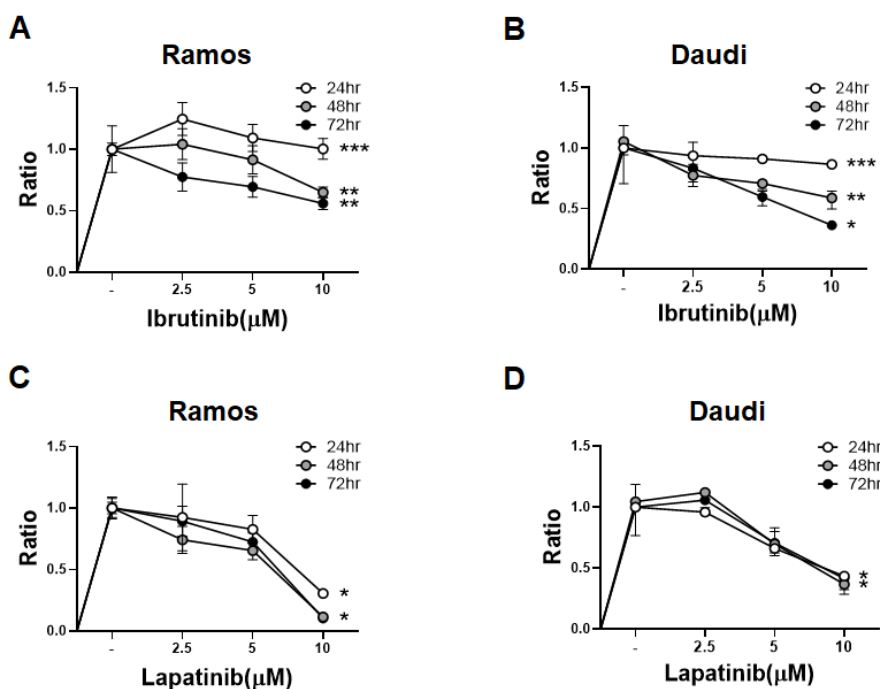
Figure 1. Cell proliferation decreases in a dose-dependent manner at 24, 48, and 72 hours. (A) Ramos cells were treated with varying concentrations of Ibrutinib over 24, 48, and 72 hours, demonstrating a dose-dependent decrease in cell proliferation, as evaluated by the Cell Counting Kit-8 (CCK-8) assay. (B) Daudi cells were subjected to different concentrations of Ibrutinib for the same time intervals, revealing a dose-dependent inhibition of cell proliferation, as assessed by the CCK-8 assay. (C) In Ramos cell lines, treatment with Lapatinib at different concentrations and time points produced a dose-dependent reduction in cell proliferation, as measured by the CCK-8 assay. (D) Daudi cell lines exhibited a dose-dependent inhibition of cell proliferation when exposed to various concentrations of Lapatinib over 24, 48, and 72 hours, as determined by the CCK-8 assay. Statistical testing was conducted with two-tailed, unpaired t-tests, *$P<0.05$; **$P<0.01$; ***$P<0.001$. Error bars represent the mean± SD.
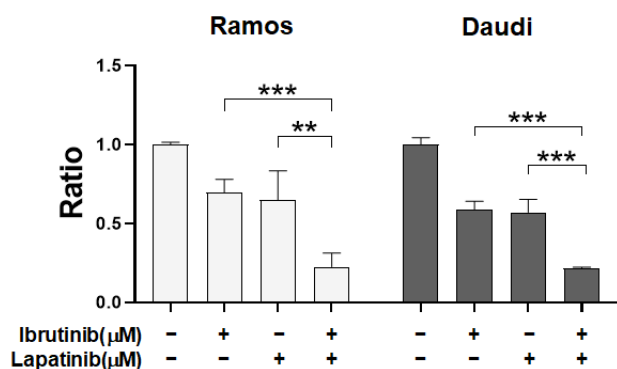

Figure 2. Synergistic therapeutic effects in Ramos and Daudi cells. Cell viability of Ramos and Daudi cells treated with a combination of Ibrutinib and Lapatinib was analyzed using the CCK-8 assay after 72 hours. In both cell lines, the dual-targeting therapy resulted in a significant reduction of over 50% compared to control and single treatment, demonstrating the pronounced synergistic therapeutic effects. Statistical testing was conducted with two-tailed, unpaired t-tests, **$P<0.01$; ***$P<0.001$. Error bars represent the mean±SD.

results strongly indicate that a combined therapeutic approach has the potential to effectively suppress the survival and proliferation of Burkitt's lymphoma cells, highlighting the presence of synergistic effects.

3. Combination of Ibrutinib and Lapatinib Induced Upregulation of Apoptosis-related Genes

Prior research has established that phosphorylated p53 activates pro-apoptotic genes, including the

Bcl-2-associated X protein (Bax) [9, 10]. In our investigation, we observed that the combined drug treatment of Ramos and Daudi cells notably increased the expression of *TP53* and *Bax* compare to Ibrutinib, leading to the apoptosis of lymphoma cells (Figure 3A, 3B). However, there was no difference between Lapatinib and combined drugs in Daudi cells. To unravel the synergistic inhibitory effects of Ibrutinib and Lapatinib in Burkitt's lymphoma, we performed flow cytometric analysis to delve into the underlying mechanisms. To confirm drug-induced cell death, we used annexin V-PI analysis. The results showed a clear combined effect of Ibrutinib + Lapatinib in the Q2 (late apoptosis) and Q3 (early apoptosis) quadrants (Figure 3C).

4. Combination of Ibrutinib and Lapatinib Induces G2/M-phase Arrest in Burkitt's Lymphoma

In order to explore the connection between the inhibition of cell proliferation and cell cycle regulation, we conducted flow cytometric analysis to evaluate the distribution of cells in different cell cycle phases. Following treatment with a combination of Ibrutinib and Lapatinib, there was a noticeable increase the Burkitt's lymphoma cells in the G2/M phase after 72
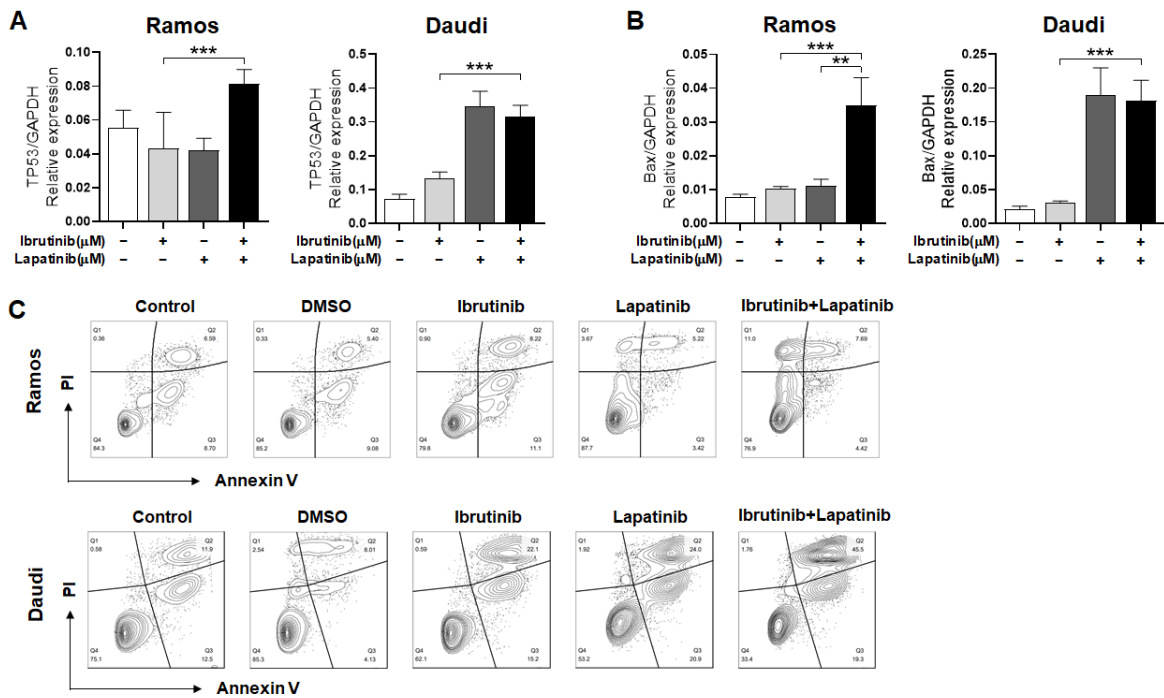
Figure 3. Induction of apoptosis by dual-targeted therapeutic combination. (A, B) Quantitative polymerase chain reaction (qPCR) analysis was performed to assess apoptotic markers following treatment with Ibrutinib and Lapatinib in Ramos and Daudi cell lines. The mRNA levels of p53, and Bax were evaluated in both cell lines following treatment with drugs, as determined by qPCR. The expression of each sample was normalized with housekeeping gene, *GAPDH*. (C) Cells were treated with individual or combinations of 5 μM of Ibrutinib with or without Lapatinib for 72 hours. The percentages of early apoptotic cells (annexin V-positive/propidium iodide [PI]-negative), live cells (annexin V-negative/PI-negative) and late apoptotic cells (annexin V-positive/PI-positive) were compared with control (dimethyl sulfoxide, DMSO). Statistical testing was conducted with two-tailed, unpaired t-tests, **$P<0.01$; ***$P<0.001$. Error bars represent the mean±SD.
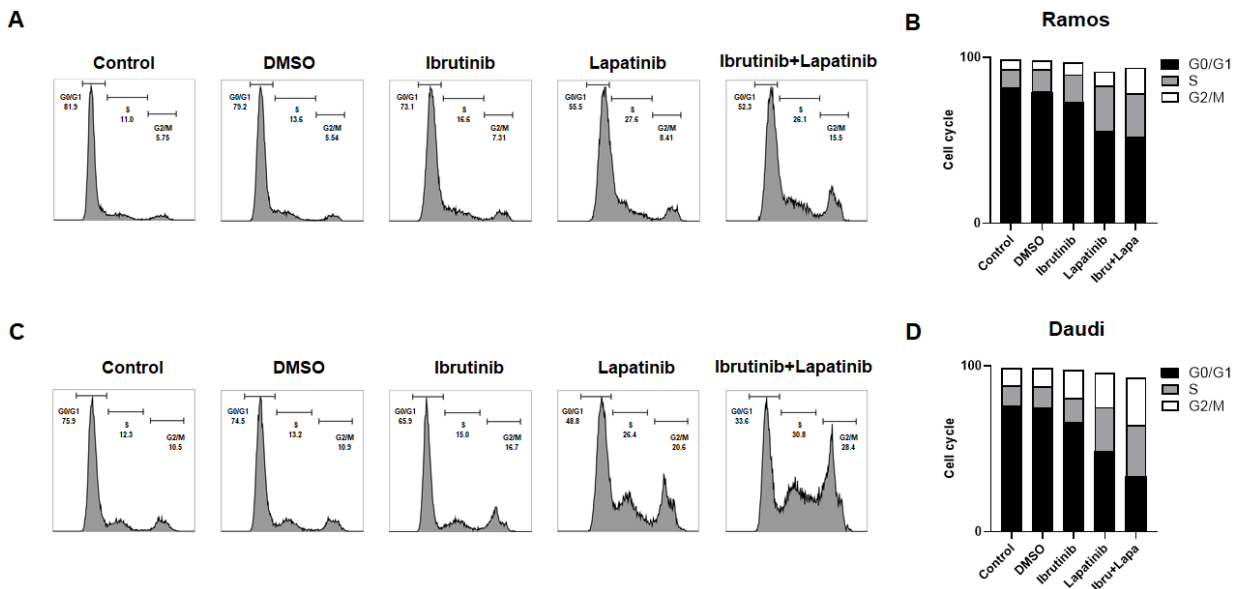

Figure 4. Combination treatment with Ibrutinib and Lapatinib resulted in an increased G2/M phase arrest, as assessed by flow cytometry analysis of cell cycle phases. (A) In the Ramos cells, the major peaks corresponded to the G0/G1 phase, S phase, and G2/M phase from left to right. (B) Ramos cells were treated with 5 μM of Ibrutinib and Lapatinib for 72 hours. (C) In the Daudi cells, the major peaks corresponded to the G0/G1 phase, S phase, and G2/M phase from left to right. (D) Daudi cells were treated with 5 μM of Ibrutinib and Lapatinib for 72 hours. The experiment was repeated in duplicate and merged data from all the experiments are shown. Statistical testing was conducted with two-tailed, unpaired t-tests. *$P<0.05$ compared with the single group.
Abbreviation: DMSO, dimethyl sulfoxide.

hours of treatment. Simultaneously, there was a corresponding decrease in the number of cells in the G0/G1 phase observed in both Ramos (Figure 4A, 4B) and Daudi cells (Figure 4C, 4D).

DISCUSSION

The findings of these results underscore the potential of Ibrutinib and Lapatinib as a combination therapy in the Burkitt's lymphoma. We observed a significant inhibition of cell proliferation in Burkitt's lymphoma cells when treated with Ibrutinib and Lapatinib individually, corroborating their efficacy as single agents (Figure 2). However, the most compelling aspect of our research emerges from the synergy demonstrated when both drugs were used concurrently.

The efficacy of Ibrutinib in B-cell malignancies is well-documented, consistent with our observations (Figure 1A, 1C). It acts on the B-cell receptor signaling pathway, a critical factor in the viability and proliferation of malignant B-cells [11]. The potent inhibition of this pathway in Burkitt's lymphoma cells, as evidenced by our results, aligns with previous research and highlights Ibrutinib's potential as a therapeutic agent [12].

Lapatinib, designed primarily for HER2-positive breast cancer, has shown remarkable success in arresting the growth of tumors driven by HER2 overexpression [13]. Our results demonstrate its efficacy in Burkitt's lymphoma cells, extending its potential applications beyond breast cancer. We have previously demonstrated the inhibition of Burkitt lymphoma through the combination of Lapatinib with other drugs [14]. This warrants further exploration of Lapatinib as a candidate for diversified cancer therapies.

The combined effects of Ibrutinib and Lapatinib on Burkitt's lymphoma cells are particularly striking. The concurrent treatment led to a notable reduction in cell proliferation, supporting the hypothesis that the simultaneous targeting of multiple intracellular pathways could result in a synergistic response. Such a combination strategy is particularly promising for Burkitt lymphoma, where rapid and aggressive treatment is imperative.

Additionally, our study sheds light on the potential mechanistic underpinnings of this combination therapy. Phosphorylated p53 activation, known to induce proapoptotic genes such as *Bax* [9], was evident in the presence of both drugs (Figure 3A, 3B). This molecular response provides insight into the apoptosis-driven mechanism behind the observed reduction in cell proliferation. However, we did not investigate apoptosisassociated proteins like caspase-3 and PARP [15-17]. Additional research is warranted to delve further into this aspect.

Furthermore, we observed G2/M-phase cell cycle arrest in Burkitt's lymphoma cells upon combined Ibrutinib and Lapatinib treatment (Figure 4). This disruption in cell cycle progression may contribute to the reduced cell proliferation observed in our experiments [18-20].

In conclusion, the results of this investigation indicate that a combination of Ibrutinib and Lapatinib holds significant promise as a treatment approach for Burkitt's lymphoma. The synergistic effect observed in our experiments, combined with the upregulation of apoptosis-related genes and cell cycle arrest, emphasizes the potential of this dual-agent therapy. Nonetheless, comprehensive in vivo investigations and clinical trials are necessary to substantiate these results and evaluate the safety and effectiveness of this combined treatment approach for Burkitt's lymphoma within a clinical context. If successful, this approach could pave the way for improved outcomes and novel therapeutic strategies for this aggressive malignancy.

요 약

버킷 림프종(Burkitt's lymphoma)은 B-세포에서 발생하는 비호지킨 림프종의 한 형태로, 빠른 성장과 면역계 장애와 관련된 특성으로 인해 약물이 투여되지 않으면 생존율이 감소

하거나 나쁜 예후로 이어질 수 있다. Food and Drug Administration (FDA) 승인된 약물의 최적 활용, 안전한 약물을 병용하는 방법은 시간과 비용 절감을 가능하게 한다. 이 접근법은 새로운 치료법 개발을 하기 보다는 기존에 FDA 승인된 약물을 적응증이 다른 환자에게 빠르게 접근할 수 있는 가능성을 제공한다. 이 연구는 BTK를 표적으로 하는 이브루티닙(Ibrutinib)과 EGFR/HER2를 표적으로 하는 라파티닙(Lapatinib) 병용 치료 전략의 잠재력을 확인하였다. 버킷 림프종의 잘 알려진 Ramos 및 Daudi 세포주가 이 연구에 활용되어 이 병합 치료의 영향을 밝히는 역할을 하였다. 이브루티닙과 라파티닙의 병용 치료가 단일 약물 대비 세포 증식을 상당히 억제하는 것을 보여주었다. 또한 병용 치료가 세포 사멸을 유도하고 S 및 G2/M 단계에서 세포주기 중단을 유발하는 것을 관찰하였다. 이 접근법은 약물 개발 일정을 간소화하는 데 그치지 않고 이미 존재하는 자원의 활용을 극대화하는 것을 의미한다.

Acknowledgements: None

Conflict of interest: None

Author's information (Position): Yang CE, Graduate student; Kim SB, Graduate student; Jeong Y, Master graduate student; Lim JY, Professor.

Author Contributions

- Conceptualization: Yang CE.
- Data curation: Yang CE, Kim SB, Jeong Y.
- Formal analysis: Yang CE, Kim SB.
- Methodology: Jeong Y.
- Validation: Lim JY.
- Writing - original draft: Lim JY, Yang CE.
- Writing - review & editing: Yang CE, Lim JY.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

ORCID

Chae-Eun YANG	https://orcid.org/0009-0001-6550-7617
Se Been KIM	https://orcid.org/0009-0001-7932-6964
Yurim JEONG	https://orcid.org/0009-0001-2658-357X
Jung-Yeon LIM	https://orcid.org/0000-0001-5903-8810

REFERENCES

1. Molyneux EM, Rochford R, Griffin B, Newton R, Jackson G, Menon G, et al. Burkitt's lymphoma. Lancet. 2012:379:1234-1244. https://doi.org/10.1016/s0140-6736(11)61177-x
2. López C, Burkhardt B, Chan JKC, Leoncini L, Mbulaiteye SM, Ogwang MD, et al. Burkitt lymphoma. Nat Rev Dis Primers. 2022:8:78. https://doi.org/10.1038/s41572-022-00404-3
3. Salles G, Barrett M, Foà R, Maurer J, O'Brien S, Valente N, et al. Rituximab in B-cell hematologic malignancies: a review of 20 years of clinical experience. Adv Ther. 2017:34:2232-2273. https://doi.org/10.1007/s12325-017-0612-x
4. Xue C, Wang X, Zhang L, Qu Q, Zhang Q, Jiang Y. Ibrutinib in B-cell lymphoma: single fighter might be enough? Cancer Cell Int. 2020:20:467. https://doi.org/10.1186/s12935-020-01518-y
5. Kim E, Hurtz C, Koehrer S, Wang Z, Balasubramanian S, Chang BY, et al. Ibrutinib inhibits pre-BCR⁺ B-cell acute lymphoblastic leukemia progression by targeting BTK and BLK. Blood. 2017; 129:1155-1165. https://doi.org/10.1182/blood-2016-06-722900
6. Johnston SR, Leary A. Lapatinib: a novel EGFR/HER2 tyrosine kinase inhibitor for cancer. Drugs Today (Barc). 2006:42:441-453. https://doi.org/10.1358/dot.2006.42.7.985637
7. Opdam FL, Guchelaar HJ, Beijnen JH, Schellens JH. Lapatinib for advanced or metastatic breast cancer. Oncologist. 2012:17:536-542. https://doi.org/10.1634/theoncologist.2011-0461
8. Chipuk JE, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD, Schuler M, et al. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. Science. 2004;303:1010-1014. https://doi.org/10.1126/science.1092734
9. Basu A, Haldar S. The relationship between Bcl2, Bax and p53: consequences for cell cycle progression and cell death. Mol Hum Reprod. 1998;4:1099-1109. https://doi.org/10.1093/molehr/4.12.1099
10. Herman SE, Mustafa RZ, Gyamfi JA, Pittaluga S, Chang S, Chang B, et al. Ibrutinib inhibits BCR and NF-κB signaling and reduces tumor proliferation in tissue-resident cells of patients with CLL. Blood. 2014;123:3286-3295. https://doi.org/10.1182/blood-2014-02-548610
11. Jeong Y, Kim SB, Yang CE, Yu MS, Choi WS, Jeon Y, et al. Overcoming the therapeutic limitations of EZH2 inhibitors in Burkitt's lymphoma: a comprehensive study on the combined effects of MS1943 and Ibrutinib. Front Oncol. 2023;13:1252658. https://doi.org/10.3389/fonc.2023.1252658
12. Bilancia D, Rosati G, Dinota A, Germano D, Romano R, Manzione L. Lapatinib in breast cancer. Ann Oncol. 2007;18 Suppl 6:vi26-30. https://doi.org/10.1093/annonc/mdm220
13. Kim SB, Yang CE, Jeong Y, Yu M, Choi WS, Lim JY, et al. Dual targeting of EZH2 degradation and EGFR/HER2 inhibition for enhanced efficacy against Burkitt's lymphoma. Cancers (Basel). 2023;15:4472. https://doi.org/10.3390/cancers15184472
14. Los M, Mozoluk M, Ferrari D, Stepczynska A, Stroh C, Renz A, et al. Activation and caspase-mediated inhibition of PARP: a molecular switch between fibroblast necrosis and apoptosis in death receptor signaling. Mol Biol Cell. 2002;13:978-988. https://doi.org/10.1091/mbc.01-05-0272
15. Taylor WR, Stark GR. Regulation of the G2/M transition by p53.

Oncogene. 2001;20:1803-1815. https://doi.org/10.1038/sj.onc.1204252

16. Srivastava N, Saxena AK. Caspase-3 activators as anticancer agents. Curr Protein Pept Sci. 2023;24:783-804. https://doi.org/10.2174/1389203724666230227115305

17. Cui Q, Yu JH, Wu JN, Tashiro S, Onodera S, Minami M, et al. P53-mediated cell cycle arrest and apoptosis through a caspase-3- independent, but caspase-9-dependent pathway in oridonin-treated MCF-7 human breast cancer cells. Acta Pharmacol Sin. 2007;28:1057-1066. https://doi.org/10.1111/j.1745-7254.2007.00588.x

18. Nair R, Roden DL, Teo WS, McFarland A, Junankar S, Ye S, et al. c-Myc and Her2 cooperate to drive a stem-like phenotype with poor prognosis in breast cancer. Oncogene. 2014;33:3992-4002. https://doi.org/10.1038/onc.2013.368

19. Park C, Cha HJ, Lee H, Hwang-Bo H, Ji SY, Kim MY, et al. Induction of G2/M cell cycle arrest and apoptosis by Genistein in human bladder cancer T24 cells through inhibition of the ROS-dependent PI3k/Akt signal transduction pathway. Antioxidants (Basel). 2019;8:327. https://doi.org/10.3390/antiox8090327

20. Xia W, Spector S, Hardy L, Zhao S, Saluk A, Alemane L, et al. Tumor selective G2/M cell cycle arrest and apoptosis of epithelial and hematological malignancies by BBL22, a benzazepine. Proc Natl Acad Sci U S A. 2000;97:7494-7499. https://doi.org/10.1073/pnas.97.13.7494