Master of Science

Phosphodiesterase 5 inhibitors for drug repositioning in a mouse model of chemotherapy-induced cognitive impairment

The Graduate School
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HYUN-JI KIM

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Supervisor : EUN-JAE LEE

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HYUN-JI KIM

Department of Medical Science
University of Ulsan, Korea
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Exploring the potential of Phosphodiesterase 5 inhibitors inhibitors for drug repositioning in a mouse model of chemotherapy-induced cognitive impairment.

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Committee Chair Seong Joo Lee (Sign)

Committee Eun-Jae Lee (Sign)

Committee Yang Sik Kim (Sign)

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University of Ulsan

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Abstract

Given the constant progress in cancer diagnostics and therapeutic interventions, the life expectancy of cancer patients, including breast cancer, which ranks as the second most prevalent cancer among women in our country, has been steadily increasing. In fact, the 5-year survival rate for breast cancer now surpasses 90%, prompting a surge in interest regarding the patients' quality of life. Among the various factors influencing quality of life, cognitive function stands out as a significant component. Studies indicate that approximately 35-70% of breast cancer patients experience cognitive impairments subsequent to undergoing antineoplastic therapy, commonly referred to as Chemotherapy-Induced Cognitive Impairment (CICI) or "chemobrain." The principal culprit behind cognitive impairments in cancer patients is believed to be certain chemotherapeutic agents, such as Paclitaxel (PTX). While the precise mechanisms underlying the onset of cognitive impairments due to cancer treatments remain elusive, several proposed mechanisms include oxidative stress, disruption of the blood-brain barrier, and heightened inflammatory responses.

The objectives of this research endeavor were twofold: 1) to establish a CICI rodent model utilizing PTX, the quintessential chemotherapeutic agent implicated in breast cancer-induced cognitive impairments, and 2) to propose a novel therapeutic approach for CICI by activating Nitric Oxide Synthase and accumulating cGMP using a PDE5 inhibitor, known for its anti-inflammatory and antioxidant effects. The use of PDE5 inhibitors is advantageous owing to their widespread application in the treatment of erectile dysfunction, offering favorable pharmacokinetic properties and a favorable side effect profile.

To achieve these objectives, PTX was intraperitoneally administered to 8-month-old rodents, followed by a battery of behavioral experiments encompassing the Open Field Test, Novel Object Recognition Test, and Morris Water Maze. Preliminary findings revealed a mild proclivity towards cognitive impairments in PTX-treated animals compared to those subjected to sham injections, although the effect magnitude was not substantial. Additionally, assessment of protein levels demonstrated a heightened inclination towards inflammation and oxidative stress in the PTX-treated group. However, the administration of a PDE5 inhibitor as a therapeutic

intervention for ameliorating cognitive impairments failed to restore performance in the behavioral experiments. Moreover, at the protein level, the PDE5 inhibitor did not significantly mitigate the inflammation and oxidative stress observed in the CICI model, despite an evident tendency towards increased inflammation and oxidative stress in the CICI model compared to the sham treatment group. Consequently, it is postulated that while inflammation and oxidative stress may contribute to the mechanism underlying PTX-induced cognitive impairments, the PDE5 inhibitor did not exert sufficient inhibitory effects in this context. These findings may be attributed to the possibility of relatively mild cognitive impairments in the CICI model employed in this study, as well as potential inadequacies in the dosage or administration method of the PDE5 inhibitor. As our research centers on investigating therapeutic agents for cancer patients, future investigations may involve modeling with rodents harboring cancer cells and exploring alternative strategies, such as modifying the dosage or administration method of the PDE5 inhibitor.

In summary, this study successfully established a CICI rodent model induced by antineoplastic therapy and assessed the potential of PDE5 inhibitors as a novel treatment option. Although the efficacy of the PDE5 inhibitor was not fully evident in this study, it is anticipated that further research and subsequent refinements will lead to advancements in this field.

Ⅰ. Introduction

1.1 Background and Objectives of the Study

Peripheral nervous system side effects such as loss of balance, clumsiness, difficulty in fine motor tasks like picking up objects and buttoning clothing, walking problems, and hearing loss are well-known after-effects of cancer treatment. Recently, it has been recognized that central nervous system side effects, particularly cognitive impairment, also occur, but the underlying mechanisms of these side effects are not well understood. According to statistical data, South Korea has a relatively high cancer survival rate compared to the United States and Europe, with reports indicating that 7 out of 10 patients survive for more than five years. Cancer patients experience various side effects after receiving anticancer treatment, including physical fatigue, pain, cognitive decline, and sleep disturbances, as well as psychological issues such as anxiety, depression, and fear, and social challenges such as childcare and returning to work. These factors significantly diminish the quality of life for patients. Among the many side effects that affect quality of life, cognitive function plays a crucial role. The cognitive impairments experienced after chemotherapy are referred to as chemotherapy-induced cognitive impairment (CICI), also known as "chemobrain" or "chemofog." The symptoms of chemobrain include a mild cognitive impairment characterized by mental fog, reduced concentration, difficulty in acquiring new information, and problems with short-term memory. These impairments significantly impact the quality of life. Quality of life refers to the level of well-being and happiness, and cognitive function is an essential factor within it. However, rehabilitation therapies for these long-term effects have a low rate of approximately 6%. Therefore, we conducted this study to investigate the recovery of cognitive impairments, which are crucial for living a meaningful life.

Among various types of cancer, we chose breast cancer for this study because it is the second most common cancer among Korean women and has a high survival rate of over 91.2% for more than five years, with a continuous increase in the number of patients. According to several

statistical reports, the incidence of breast cancer is steadily rising, and as of 2018, the overall survival rate for all age groups exceeds 90%. Many research papers indicate that 15–50% of breast cancer patients experience cognitive impairment. The representative therapeutic agents for breast cancer are Doxorubicin and Paclitaxel, and we conducted our research using Paclitaxel. The reason for choosing Paclitaxel is that Doxorubicin has been reported to cause not only neurotoxicity but also cardiotoxicity. In a previous study, Paclitaxel, which does not cross the blood–brain barrier, was reported to induce cognitive impairments in rodents. In this study, we aimed to establish a behavioral experiment based on previous studies on cognitive impairments and provide a more diverse phenotypic characterization. Furthermore, although the exact mechanisms of CICI are not yet fully understood, several potential mechanisms have been suggested, including oxidative stress, blood–brain barrier disruption, cytokine dysregulation mediating immune and inflammatory responses, insufficient DNA repair, DNA damage, telomere shortening, altered hormone levels, and neuronal genetic polymorphisms. This study aimed to elucidate that oxidative stress and cytokine dysregulation mediating immune and inflammatory responses are the key mechanisms of chemobrain.

Moreover, we aimed to evaluate the efficacy of a phosphodiesterase type 5 (PDE5) inhibitor, known for its anti–inflammatory and antioxidant effects, in alleviating the phenotype of CICI induced by Paclitaxel. PDE5 inhibitors are widely used as medications for erectile dysfunction, and there are research findings indicating that they improve cognitive function. Studies have also shown the efficacy of PDE5 inhibitors in alleviating cognitive impairments in patients with dementia or other cognitive disorders. Therefore, we used Udenafil, one of the PDE5 inhibitors, to investigate its potential in alleviating cognitive impairments induced by CICI.

Ⅱ. Research Methods

2.1 Animals

The animals used in the study were 8-week-old C57BL/6J mice purchased from The Jackson Laboratory. The mice were housed in groups of four per cage and maintained under a 12:12 light-dark cycle. All experiments were conducted during the light phase, and the mice's body weights were measured daily.

2.2 Drug Administration

Paclitaxel (PTX, CAS 33069-62-4) was dissolved in Kolliphor® EL: ethanol (1:1) to create a stock solution with a concentration of 6 mg/ml. Before use, the stock solution was diluted to a concentration of 2 mg/ml in 0.9% NaCl solution and administered intraperitoneally at a dose of 20 mg/kg. The control group received an equivalent amount of Kolliphor® EL: ethanol (1:1) as the stock solution, which was diluted to a concentration of 33.3% Kolliphor® EL: ethanol in 0.9% NaCl solution before administration. Udenafil (Republic of Korea, Dong-A Pharmaceutical) was dissolved in a 0.9% NaCl solution containing 20% cyclodextrin to achieve a final concentration of 0.6 mg/ml and administered intraperitoneally at a dose of 3 mg/kg. The control group received an equivalent amount of 20% cyclodextrin.

After the administration of Udenafil, Paclitaxel was administered 20 minutes later.

2.3 Behavioral Experiments

Novel Object Recognition Test (NORT): This behavioral analysis was conducted to observe aspects of learning and memory in mice. The test consisted of a habituation trial, a training trial, and a test trial conducted over three days. In the habituation trial, the mice were placed in an empty arena for 30 minutes to familiarize them with the NORT. In the training trial, two identical objects were placed, and the mice were allowed to explore the objects for 15 minutes. Finally, in the test trial, one of the objects was replaced with a novel object.

Morris Water Maze Test (MWM): The Morris water maze is a behavioral analysis test related to spatial learning and memory. Mice were placed in a large circular swimming pool and required to escape from the water to a hidden platform using spatial memory based on cues on the walls. After five days of training trials to memorize the platform's location, a probe trial was conducted to assess how quickly and accurately the mice navigated to the former platform location when the platform was removed.

2.4 Protein Analysis

Proteins were extracted by grinding tissue in lysis buffer (RIPA: 50 mM Tris, pH 7.8, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.5% SDS, and 5 mM EDTA) supplemented with protease inhibitors (1 mg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride) and phosphatase inhibitors (2.5 mM sodium pyrophosphate, 1 mM Na3VO4). Equal amounts of protein were loaded onto a 10% gel, separated by SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis), and transferred to polyvinylidene difluoride membranes.

In the analysis of inflammation markers, Interleukin 1-beta (R&D Systems, AF-401-NA), IL-6 (Abcam, ab229381), and TNF alpha (Abcam, ab9739) were employed. As for oxidative stress markers, DNP (Bethyl, A150-117A) and Nitrotyrosine (Enzo, ADI-905-763) were utilized. Western blotting was performed using an appropriate secondary antibody for each primary antibody, with options including mouse, rabbit, and goat antibodies.

Ⅲ. Results and Analysis

3.1 Hypothesis

In this study, we hypothesized that the administration of Paclitaxel, a chemotherapy drug for breast cancer, would exacerbate inflammation and oxidative stress in the hippocampus, a region responsible for cognitive function, leading to cognitive impairment. Additionally, we hypothesized that the PDE5 inhibitor Udenafil, which possesses anti-inflammatory and antioxidant properties, would alleviate cognitive impairment in the Paclitaxel-induced CICI model (Fig. 1).

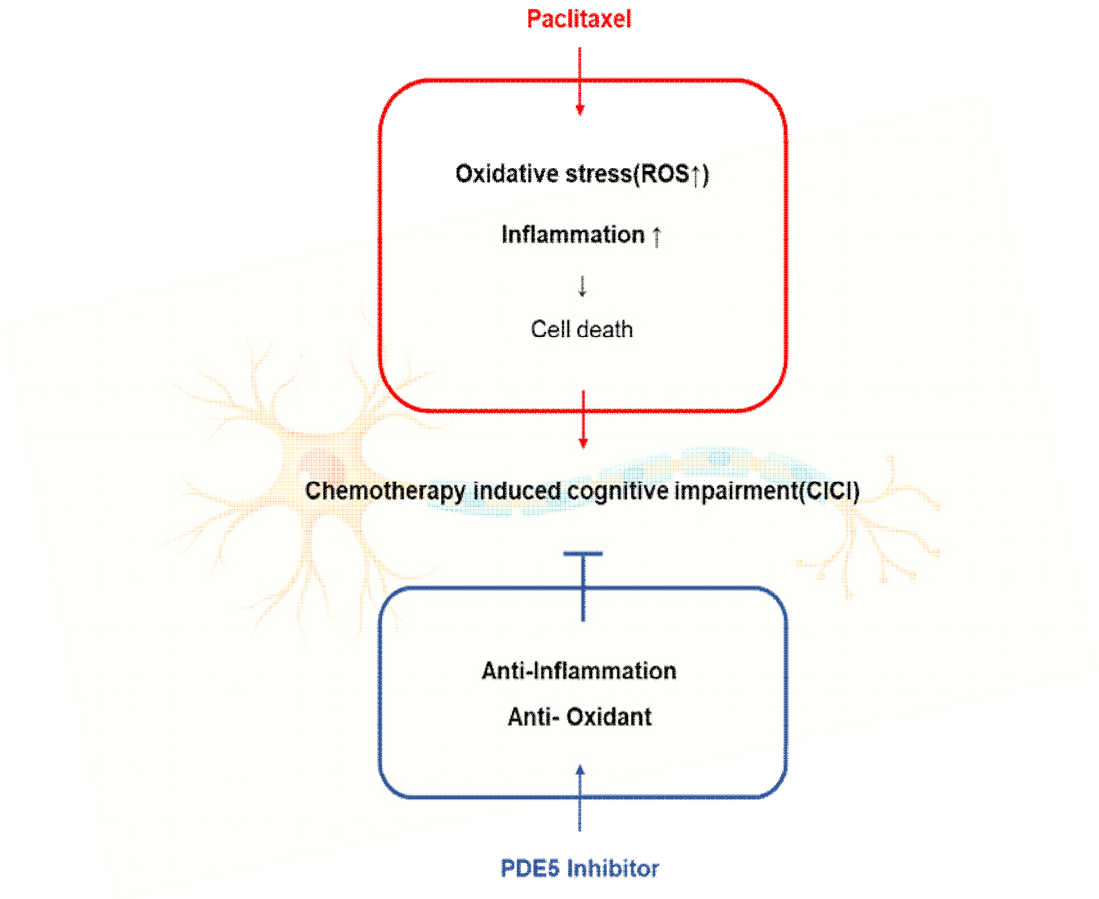

Figure 1. After inducing chemotherapy-induced cognitive impairment (CICI) in mice using Paclitaxel, a breast cancer therapeutic agent, we aimed to evaluate whether PDE5 inhibitor alleviate the phenotype of the model.

3.2 Induction of the Paclitaxel Model

We utilized 8-week-old adult C57BL/6J mice and administered intraperitoneal injections of Paclitaxel (PTX) at a dose of 20 mg/kg body weight (equivalent to human dosage) for a total of 12 injections over a period of 4 weeks. Subsequently, we conducted a one-week recovery period with handling before proceeding to behavioral experiments (Fig. 2a). The condition of the model mice was monitored by daily measurement of body weight, and once the body weight returned to normal during the recovery period after the initial decrease following injection, the behavioral experiments were conducted (Fig. 2b). For assessing cognitive function, we performed the Novel Object Recognition Test (NORT) and Morris Water Maze (MWM) experiments. NORT, conducted over two days, involved placing two identical objects on the first day and replacing one of the objects with a novel object on the second day to measure preference for the novel object (Cognition, Recognition memory). In our study, the PTX model demonstrated a significant decrease in preference for the novel object in the NORT (Fig. 2c). This suggests that the PTX group exhibited impaired recognition memory compared to the control group, indicating the progression of chemotherapy-induced cognitive impairment (CICI). To further investigate this cognitive function, we conducted the Morris Water Maze (MWM) test.

MWM involves placing the mouse in a pool and training them to find an escape platform over a week, followed by a probe test to assess spatial memory and learning. Although the overall graph did not show statistical significance, our results indicated a tendency for decreased memory in the PTX group compared to the control group. As mentioned earlier in the background, CICI in humans also manifests as mild cognitive impairment in only a subset of patients, characterized by reduced attentiveness, decreased concentration, and short-term memory problems, rather than severe cognitive impairment. Thus, our model successfully induced CICI (Fig. 2d).

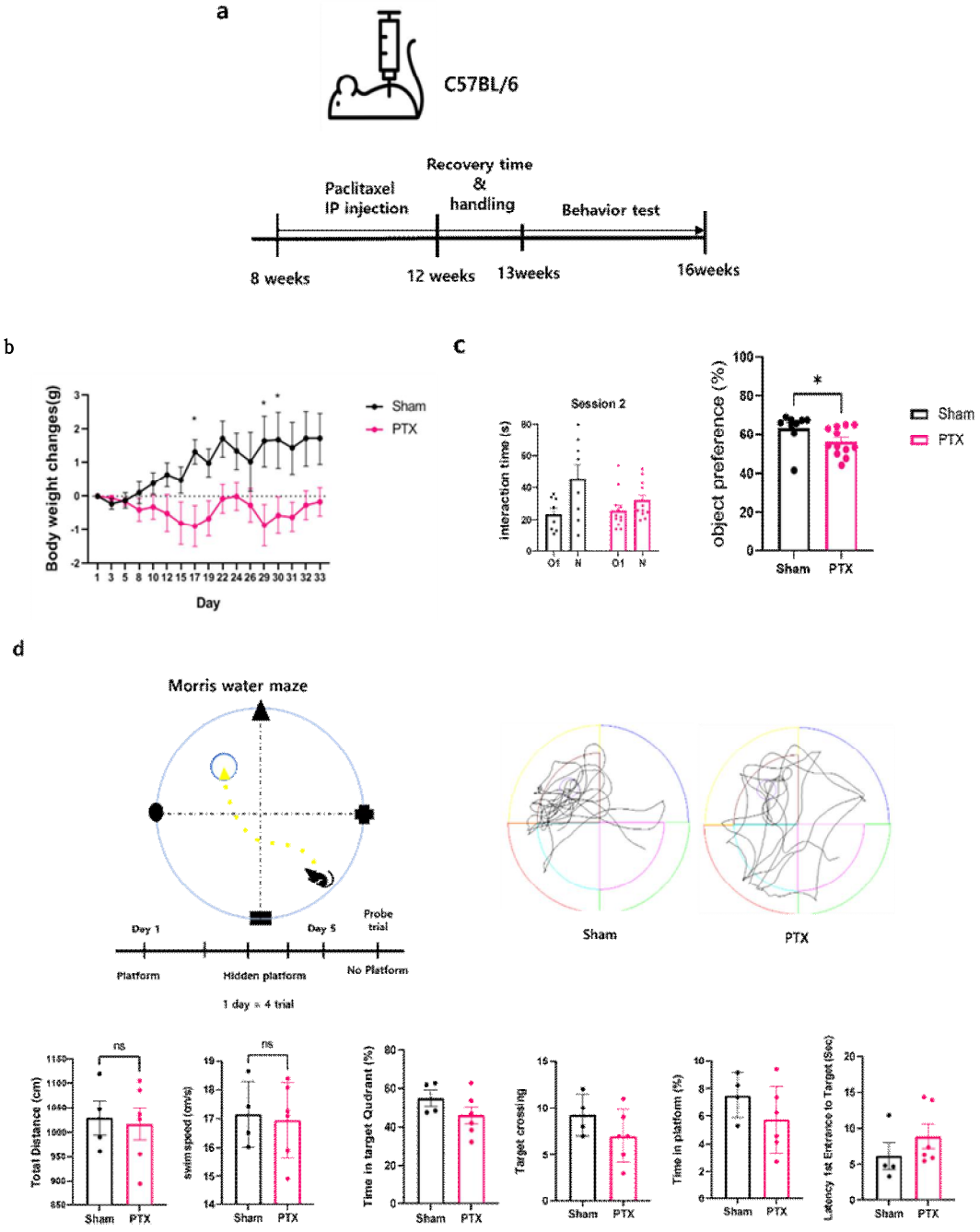

Figure 2. Chemotherapy-induced cognitive impairment (CICI) model induction using Paclitaxel in mice.

(a) CICI modeling was performed using the corresponding protocol.
(b) Body weight decreased in the PTX group compared to the Sham group. Two-way ANOVA followed by Šídák's multiple comparisons test, *P≤0.05.
(c) No significant differences were observed in the Open Field Test. T-test.
(d) Novel Object Recognition Test. T-test, *P=0.0497.
(e) Morris Water Maze was conducted, and although there was a trend towards worsened cognitive impairment in the PTX group compared to the Sham group, no statistical significance was observed. T-test.

3.3 Rescue Experiment in Induced CICI Model

To verify the hypothesis that PDE5 inhibitor (Udenafil), which has demonstrated therapeutic and preventive effects in other cognitive impairment-related diseases, has a therapeutic effect in CICI observed in the previous Paclitaxel model behavioral experiments, the following experiments were conducted. Additionally, to characterize the phenotype of the CICI model with mild cognitive impairment, the cognitive-related behavioral experiment protocol was divided into two difficulty levels. Adult C57BL/6J mice at 8 weeks of age were used, and they received intraperitoneal injections of PTX at a dose of 20 mg/kg body weight (equivalent to the human dose) for a total of 12 times over 4 weeks. Prior to Paclitaxel injection, Udenafil was administered at a dose of 3 mg/kg body weight daily for 30 days. Daily measurements of body weight were taken to monitor the condition, and during the treatment period, the PTX/VEH and PTX/Ude groups showed an appropriate weight loss and exhibited recovery in body weight during the week following the injections. Handling was conducted during the recovery period (Fig. 3a, b). Similar patterns were observed as in the previous experiments, and in the NORT, the PTX/VEH group showed a decreased preference for the novel object. Although the PTX/Ude group exhibited a tendency towards increased preference compared to the PTX/VEH group, no statistical significance was observed (Fig. 3c). In the MWM, no significant differences were observed among the groups (Fig. 3d).

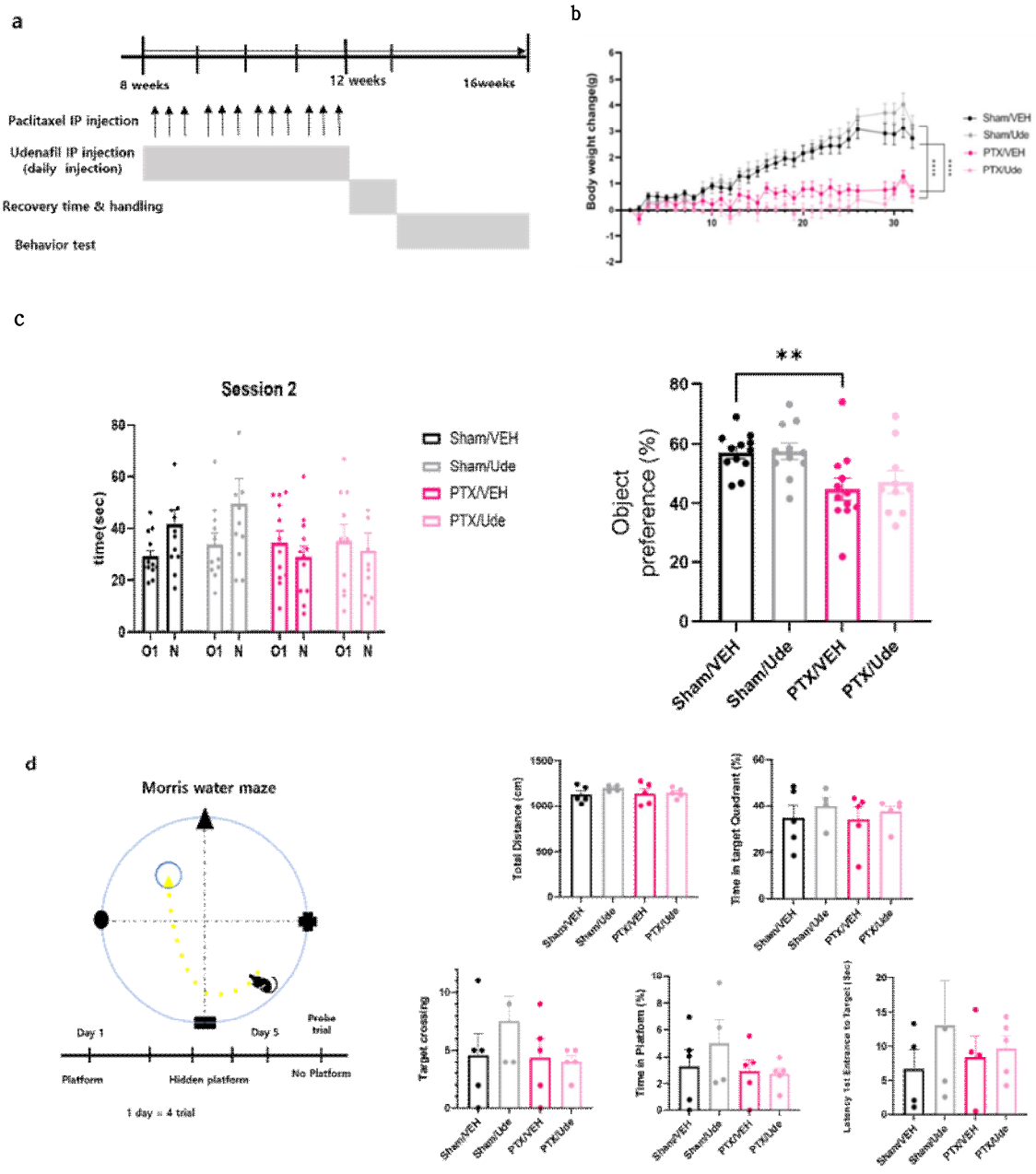

Figure 3. Treatment of the Paclitaxel-induced CICI mouse model with PDE 5 inhibitor (Udenafil).

(a) Rescue experiments using the PDE5 inhibitor were conducted following the specified protocol for the CICI model.
(b) In the groups receiving PTX injections, a decrease in body weight was observed compared to the Sham group, while no significant difference was observed in the rescue model treated with the PDE5 inhibitor. Two-way ANOVA followed by Šídák's multiple comparisons test, ****P≤0.0001
(c) Novel Object Recognition Test. T-test, **P≤0.01
(d) In the Morris Water Maze, no significant differences were observed among the groups. T-test.

3.4 Rescue Experiment with Modified Difficulty Levels in Behavioral Testing

Since the cognitive impairment observed in the CICI model itself is mild, it is possible that no phenotype is evident when using the less challenging Morris Water Maze (MWM) protocol. Therefore, we modified the protocol to increase the difficulty level and performed the experiments. Instead of starting with a visible platform on the first day, we used a hidden platform to make it more challenging from the beginning, and we reduced the number of training trials from four to three per day to shorten the overall training period (Figure 4a). The changes in MWM results across different difficulty levels were not significant. When the higher difficulty protocol was used, the PTX/VEH group showed a tendency for impaired cognitive function, similar to the results obtained under the easier conditions. However, there was no significant difference between the PTX/Ude group and the PTX/VEH group (Figure 4b). Based on these results, it appears that Udenafil does not act as a therapeutic agent in Paclitaxel-induced CICI, unlike in other cognitive disorders.

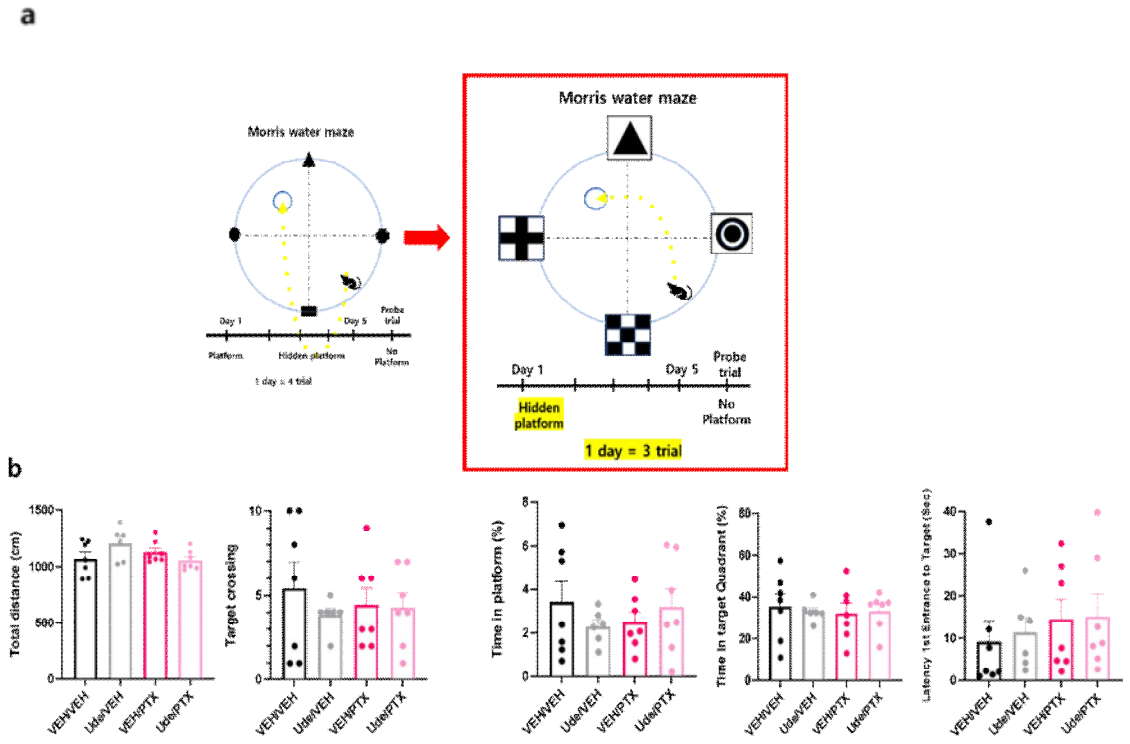

Figure 4. Modified Morris Water Maze Protocol in Udenafil and PTX-induced Model

(a) We modified the Morris Water Maze protocol to increase the difficulty level compared to the original protocol.
(b) When Morris Water Maze was conducted, the groups injected with PTX showed a tendency for worsened cognitive impairment compared to the Sham group, while the rescue model using the PDE5 inhibitor did not show a significant difference. However, there was no statistically significant difference even among the groups injected with PTX. T-test.

3.5 Protein Analysis in the Hippocampus

3.5-1 Analysis of Inflammatory Markers

In terms of protein levels, we examined three inflammatory markers in the CICI model: Interleukin-1 beta (IL-1β), Interleukin-6 (IL-6), and TNF alpha. Comparing the PTX/VEH group with the Sham/VEH group, we observed an increasing trend in IL-6 and TNF alpha levels in the PTX/VEH group, while a weak decreasing trend was observed in the PTX/Ude group. However, no statistically significant differences were observed (Fig. 5a).

3.5-2 Analysis of Oxidative Stress Markers

We also analyzed oxidative stress markers, specifically DNP and nitrotyrosine. Comparing the PTX/VEH group with the Sham/VEH group, we observed an increasing trend in DNP and nitrotyrosine levels in the PTX/VEH group. However, the PTX/Ude group did not show significant restoration of these markers (Fig. 5b). These results suggest that inflammation and oxidative stress may be involved in the mechanism underlying Paclitaxel-induced cognitive impairment. However, it appears that the PDE5 inhibitor is not sufficiently able to alleviate these effects.

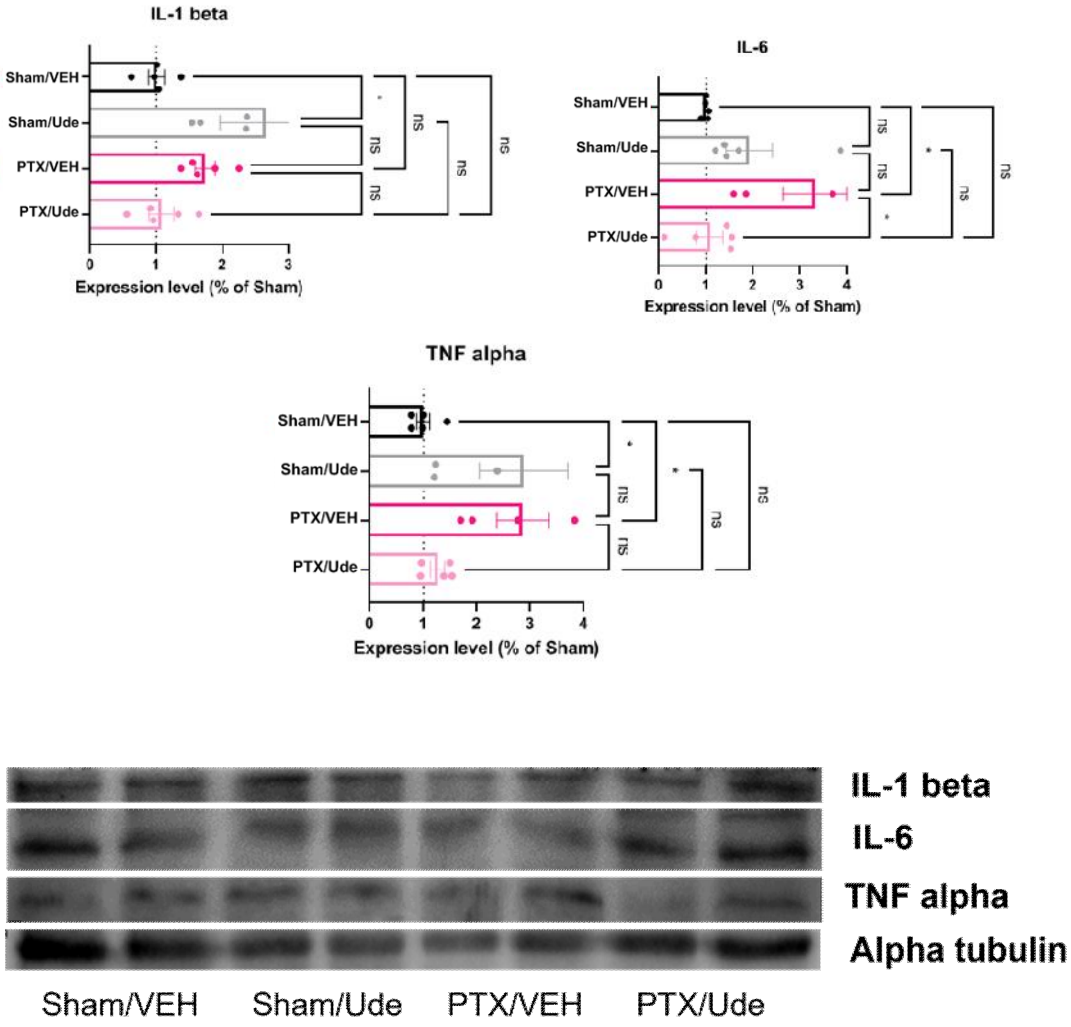

Figure 5a. Protein analysis was conducted in the hippocampus, a region involved in cognitive function. In the group that received Paclitaxel injections, an increase in inflammation markers was observed. After administering Paclitaxel (20mg/kg, 12 times) and PDE5 inhibitor (3mg/kg, 30 times), protein analysis was performed two weeks later. Statistical analysis was conducted using a two-way ANOVA followed by Sidik's multiple comparisons test, and statistical significance was indicated as *P≤ 0.05.

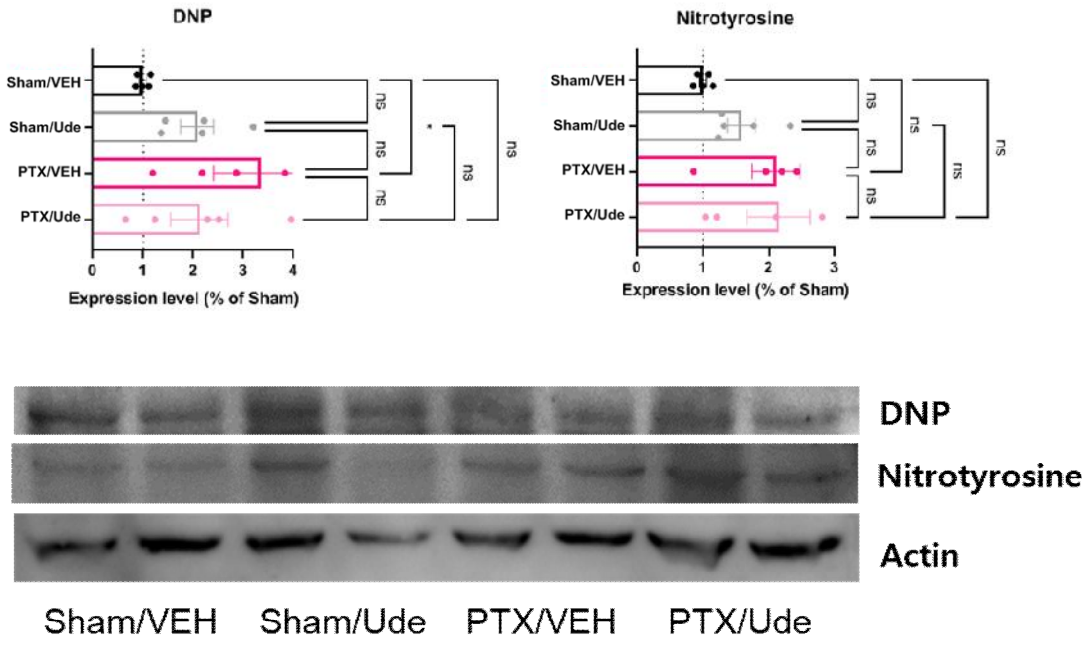

Figure 5b. In the context of cognitive function, protein analysis was conducted in the hippocampus. In the group that received Paclitaxel injections, there was an observed increase in oxidative stress markers. After administering Paclitaxel (20mg/kg, 12 times) and PDE5 inhibitor (3mg/kg, 30 times), protein analysis was performed two weeks later. Statistical analysis was carried out using a two-way ANOVA followed by Sidik's multiple comparisons test, with a significance level of $*P \leq 0.05$.

Ⅳ. Discussion

In this study, we aimed to establish a CICI (Chemotherapy-induced Cognitive Impairment) mouse model using Paclitaxel, the most representative chemotherapy for breast cancer, and explore the potential of repurposing a widely used erectile dysfunction medication, a PDE5 inhibitor, as a novel therapeutic approach for CICI. Drug repurposing offers the advantage of using known drugs, which have established safety profiles and known side effects, thereby reducing costs and time required for development.

We considered the reasons why our study did not fully align with our hypothesis. Firstly, in human cancer treatment, chemotherapy can be classified into two categories: chemo-treatment using drugs and hormonal treatment using hormone therapy. We did not investigate whether there is a difference in cognitive function between these two patient groups, which could have helped determine if chemobrain is solely caused by the drugs. Addressing this in future research could provide valuable insights.

Secondly, it is possible that the cognitive impairment observed in our CICI model was relatively mild. Considering that we conducted this study in mice with cancer cells, there is a possibility that the presence of cancer as a disease could have weakened the blood-brain barrier (BBB) or influenced cognitive impairment through other mechanisms. It is worth exploring whether cognitive impairment would be more severe when using mice with cancer cells in future studies.

Furthermore, we did not directly confirm the biological effects of chemobrain in the Paclitaxel-induced model using tissue staining (ROS) or ELISA to assess damage. Conducting further investigations in this aspect in future research could advance our understanding of this study.

Lastly, it is possible that the dose or administration method of the PDE5 inhibitor was not optimal. The dose of the PDE5 inhibitor was based on studies in dementia mice, and our intention was to prevent cognitive impairment caused by Paclitaxel by administering it concurrently. However,

altering the injection frequency, duration, or timing could lead to different outcomes. Additionally, considering the mechanisms through which Paclitaxel and PDE5 inhibitors affect the brain, using alternative markers that are directly influenced by these treatments might yield different results.

Based on these considerations, although the effects of the PDE5 inhibitor were not sufficiently evident in this study, we anticipate further progress and improvement through subsequent research.

Ⅴ. Conclusion

In conclusion, we have successfully established a rodent model of CICI with paclitaxel, one of the main treatment of breast cancer, and evaluated the potential of PDE5 inhibitors as a novel therapeutic approach. Although the full efficacy of the PDE5 inhibitor was not conclusively demonstrated in this investigation, continued research and refinement are warranted for the progress in this area of study.

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Ⅶ. 국문 요약

암 진단과 치료 방법의 발달로 암 환자의 수명이 점차 늘어나고 있다. 많은 암 중에서 국내 여성이 가장 많이 걸리는 암 2위인 유방암의 경우 5년 생존율이 90%를 넘고 이에 따라 환자들의 삶의 질에 관한 관심이 높아지고 있다. 삶의 질에서 중요한 요소 중 하나가 인지기능이다. 연구에 따르면 유방암 환자의 35~70%가 항암치료를 받은 후 인지 장애를 호소한다. 암 환자의 인지 장애의 큰 원인으로 여겨지는 것은 Paclitaxel(PTX)과 같은 항암치료로, Chemotherapy-induced Cognitive impairment (CICI) 혹은 chemobrain이라고 한다. 항암요법으로 인한 인지 장애의 메커니즘은 아직 알려지지 않았지만 산화 스트레스와 뇌혈관 장벽 파괴, 증가한 염증반응 등 몇 가지 제안된 메커니즘이 있다.

본 연구에서는 1) 유방암 유발 인지 기능장애에 대한 가장 대표적인 항암화학요법인 PTX을 이용하여 CICI 쥐 모델을 구축하고, 2) Nitric Oxide 합성효소를 활성화하고 cGMP를 축적하여 항염증과 항산화 효과를 나타내는 PDE5 inhibitor를 CICI의 새로운 치료로 제안해 보고자 하였다. PDE5의 사용은 발기부전치료제로 널리 쓰이고 있어 생체 이용과 부작용 측면에서 큰 장점이 있다.

8개월이 된 쥐에 PTX를 복강에 주사한 후, Open Field Test, Novel Object Recognition Test, Morris water maze 등의 행동 실험을 시행하였다. 먼저 PTX 주사 동물은 sham 주사 동물에 비해, 인지기능 장애의 약한 경향을 보였으나 그 정도는 강하지 않았다. 또한 단백질 수준에서 결과를 확인했을 때 염증과 산화 스트레스는 PTX를 주사한 군에서 더 높은 경향성을 나타냈다. 하지만 인지기능의 완화를 위한 치료제로 사용한 PDE5 억제제는 행동 실험을 회복시키지 않았다. 단백질 수준에서는 CICI 모델에서 염증 및 산화 스트레스가 증가하는 경향이 나타났으나 (PTX vs. sham 치료), PDE5 inhibitor가 CICI 모델의 염증 및 산화 스트레스를 유의하게 회복시키지는 못했다. 따라서 PTX의 인지기능을 일으키는 기전에 염증 및 산화 스트레스가 관여할 수는 있으나 PDE5 억제제는 이를 충분히 저해하지 못하는 것으로 생각되었다. 이런 결과는 본 연구에서 사용한 CICI 모델의 인지 장애가 다소 약했을 가능성과, PDE5 억제제의 용량이나 사용 방법이 적절하지 못했을 가능성이 있다. 암환자에서의 치료제를 연구하는 만큼 암세포를 가진 쥐에 항암치료제를 이용해 모델링을 하고 PDE5 inhibitor의 용량이나 방법에 변화를 주는 등 다른 전략을 가지고 앞으로의 연구를 진행해 볼 수 있겠다.

결론적으로 본 연구를 통해 항암치료 유발 인지 저하 생쥐모델을 성공적으로 구축했으며, 이를 이용해 PDE5 inhibitor의 신약 재창출 가능성을 검정하였다. 이번

연구에서 PDE5 inhibitor 효과가 충분히 나타나지는 않았으나 이는 향후 후속 연구를 통해 발전시켜 나갈 수 있을 것으로 기대한다.

감사의 글

마침내 짧지 않은 대학원 생활을 마무리하며 석사학위 논문 퇴고를 마칠 수 있게 되었습니다. 다니던 직장을 그만두고 학위를 얻기 위한 새로운 도전을 할 때 이 선택이 맞는 것인가 고민이 많았습니다. 여러 사람의 반대를 무릅쓰고 고집으로 왔던 대학원이었지만 너무나 좋은 교수님과 연구실 동료들을 만나 조금은 늦었다고 생각한 대학원 생활을 잘 마칠 수 있었습니다.

먼저, 제 첫 스승님이 되어주신 지도교수 이은재 교수님께 감사한 마음을 전합니다. 저에겐 교수님과의 첫 만남이 기억에 많이 남습니다. 비가 많이 오는 날 다리를 다쳐 단정하지 못한 모습으로 교수님을 만나 뵈러 갔는데 환하게 반겨주시는 모습에 이 연구실에 꼭 들어오고 싶다는 생각이 들었습니다. 감사하게도 인연이 되어 교수님의 제자로 이렇게 석사학위를 마무리할 수 있게 되었습니다. 부족한 점이 많았을 저를 학위 과정 내내 더 세심하게 지도해주려 하시고 지지해주셔서 감사합니다. 또한 심사 과정 동안 따뜻한 격려와 아낌없는 지도를 해주신 이승주 교수님, 김양식 교수님께도 감사드립니다.

대학원 생활하는 동안 실험실 생활에 적응할 수 있도록 많은 도움을 주신 김승미 선생님, 서다영 선생님께도 고마움을 전합니다. 많이 의지하고 많은 추억을 쌓을 수 있어서 대학원 생활동안 큰 힘이 되었습니다. 논문이 완성되기까지 조언해주시고 많은 도움을 주신 최린경 선생님, 신왕용 박사님께도 감사하다는 말씀을 전하고 싶습니다.

마지막으로 큰 사랑으로 항상 지지해주시고 믿어주시는 부모님께 감사의 말씀을 드립니다. 갈림길에 섰을 때 올바른 길로 갈 수 있도록 조언해주시고 철없는 딸의 선택을 믿어주셔서 감사합니다. 이러한 믿음을 바탕으로 앞으로 걸어갈 길에 자신감을 가지고 최선을 다 할 수 있을 것 같습니다.

많은 분의 가르침으로 여러 방면으로 더 발전된 사람이 된 것 같습니다. 이러한 가르침 잊지 않고 앞으로 다가올 희로애락을 즐기며 살아갈 수 있는 사람이 되겠습니다. 감사합니다.