



## Original Research

## PLAG alleviates cisplatin-induced cachexia in lung cancer implanted mice

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## ABSTRACT

Chemotherapy-induced cachexia has been a significant challenge to the successful treatment of cancer patients. Chemotherapy leads to loss of muscle, loss of appetite, and excessive weight loss, which makes these necessary treatments intolerable for most patients. Therefore, it is necessary to alleviate cachexia to successfully treat cancer patients.

In this study, tumor-implanted mouse models administered cisplatin showed rapid weight loss and reduced feeding rate by the second week of treatment, and 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG) effectively alleviated cisplatin-induced cachexia. In mice treated with cisplatin on a sacrificial day after 6 weeks, the weight of the two major leg muscles (quadriceps femoris and gastrocnemius) were reduced by up to 70%, but this muscle reduction was successfully prevented in the PLAG co-treatment group. The distribution and size of muscle fibers that appear in small units in cisplatin-treated mice were restored to normal levels by PLAG co-treatment. Furthermore, myostatin expression levels were upregulated by cisplatin, whereas myostatin decreased to normal levels with muscle recovery in the PLAG co-treated group. Tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6), which are commonly expressed in cachexia, were significantly increased in cisplatin-treated mice but were reduced to normal levels in PLAG co-treated mice. Glucose absorption, an indicator of muscle tissue activity, decreased with cisplatin treatment and recovered to normal levels with PLAG co-treatment. Overall, PLAG effectively alleviated cisplatin-induced cachexia symptoms and reduced tumor growth in tumor-implanted mice.

These findings suggest PLAG may be a promising drug to alleviate cachexia in cancer patients receiving chemotherapy.

## Abbreviation

PLAG	1-Palmitoyl-2-linoleoyl-3-acetyl-roc-glycerol
mpk	milligram per kilogram
IL-6	Interleukin-6
TNF- $\alpha$	Tumor necrosis factor- $\alpha$
CK-18	Cytokeratin-18
MuRF-1	Muscle RING-finger protein-1
IACUC	Institutional animal care and use committee

## Introduction

Despite the development of third-generation immunotherapy,

chemotherapy, which has practical and powerful anti-cancer effects, remains the first-line treatment for cancer patients. Chemotherapy eliminates abnormally growing tumor cells by inducing DNA damage, cell cycle suppression, and apoptosis [1–3]. Chemotherapy has potent anti-cancer effects, even in small doses, and is a valuable therapeutic to effectively treat cancer patients. Despite its utility as a practical and powerful anti-cancer tool, chemotherapy has serious side effects that must be resolved. Chemotherapy targets proliferating cells, which damages both cancerous tissue and healthy tissue, unlike immunotherapy and targeted anti-cancer drugs [4–7]. Chemotherapy's side effects, which include nephrotoxicity, ototoxicity, neurotoxicity, and cachexia with muscle wasting, are well known [8–15]. Among them, cachexia is regarded as a side effect that is directly related to the quality

Abbreviations: PLAG, 1-Palmitoyl-2-linoleoyl-3-acetyl-roc-glycerol.

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of life of cancer patients. Muscle wasting and/or dysfunction is accompanied by adipose tissue remodeling, weight loss, and decreased patient activity due to energy metabolic disorders [16–21]. In cases of severe cachexia symptoms, chemotherapy may be discontinued altogether [22, 23]. Despite these severe side effects, cancer patients have no alternatives to chemotherapy and there are no established therapies to mitigate these side effects. Therefore, it is necessary to develop a combination therapy that retains the anti-tumor effect of chemotherapy while simultaneously alleviating the side effects of chemotherapy.

In this study, we demonstrated that 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG) effectively reduced cachexia symptoms in mouse models with severe cisplatin-induced cachexia. The PLAG is present in the antlers of the Sika deer and can be synthesized from glycerol, palmitic acid, and linoleic acid. Our previous study demonstrated that PLAG effectively alleviated the symptoms of oral mucosal inflammation caused by chemotherapy [24, 25]. In addition, PLAG effectively alleviated symptoms of various inflammatory diseases such as acute lung injury (ALI), rheumatoid arthritis (RA), hepatitis, and gout [26–30]. Cisplatin treatment resulted in rapid weight loss and reduced food intake, which was mitigated in the PLAG co-treatment group. Furthermore, reduction in leg muscle and glucose absorption in cisplatin-treated mice was restored to normal levels through PLAG co-treatment. Additionally, the increase in myostatin and cachexia-related cytokines, such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- $\alpha$ ), was significantly reduced by the addition of PLAG. In addition to this effective mitigation of cachexia symptoms, PLAG did not interfere with the anti-tumor activity of cisplatin. Interestingly, PLAG itself has anti-tumor activity. The use of PLAG for effective mitigation of cachexia symptoms may enable continuous chemotherapy for tumor destruction, which can improve quality of life for cancer patients.

## Material & method

### Test substance (PLAG) synthesis and manufacture

PLAG was manufactured and provided by the New Drug Production Headquarters, a GMP facility of Enzychem Lifesciences Corporation (Jecheon-si, South Korea). PLAG for testing was stored and manufactured based on the material information provided by the manufacturer.

### Cell culture

A549 cells were obtained from the American Type Culture Collection (ATCC, MD, USA). Both types of cells were grown in Dulbecco's modified Eagle medium (DMEM; WelGENE, Seoul, Korea) containing 10% fetal bovine serum (HyClone, Waltham, MA, USA) and 1% antibiotics (100 mg/L streptomycin, 100 U/mL penicillin) at 37 °C in a 5% CO<sub>2</sub> atmosphere.

### Lung cancer orthotopic implantation model

Five-week-old male Balb/c nu/nu mice were obtained from NARA biotech (Yong-in, South Korea) and housed in sterile filter-topped cages. The animals (n = 6 for each treatment group) were anesthetized using isoflurane and put in a position of left lateral decubitus. A total of 2 × 10<sup>6</sup> A549 cells were suspended in a solution containing 20  $\mu$ L culture medium and 20  $\mu$ L Matrigel (BD Biosciences, NJ, USA) and were directly injected through the intercostal space into the lung to a depth of 3 mm using a 29-G needle permanently attached to a 0.5-mL insulin syringe (Becton Dickinson, NJ, USA). The mice were allowed to rest on a heating carpet until fully recovered. Starting 6 weeks after tumor cell implantation, the mice were given daily oral doses of 50 or 100 mg/kg of PLAG (n = 6 mice per group). The group in which tumors were planted and no material treatment was administered was set as a positive control group (Tumor only). And the group fed only PBS, which was the PLAG delivery, was set up separately (Tumor + PBS). The mice were sacrificed

and perfused with PBS. The lung and muscle tissue regions were extracted and fixed with 10% formaldehyde. Hematoxylin and eosin (H&E) and immunohistochemical staining was performed on the tissue sections to survey the morphology. All animal experiments were approved by the IACUC, Korea Research Institute of Bioscience & Biotechnology (approval number: KRIBB-AEC-19,207).

### Cisplatin (cDDP) delivery

Cisplatin was purchased from Sigma Aldrich (Sigma aldrich, MA, USA). The reagents were prepared according to the manufacturer's protocol and refrigerated until used. The delivery of the cDDP was performed using the IP injection method, and the dose was injected at 17:30 every Thursday. cDDP was treated with 8 mg/kg/mice.

### Cachexia monitoring

Cachexia symptoms caused by cDDP were identified as follows. Measure the weight of each individual before beginning treatment, and separate groups so that there is no variation for each group. After beginning cDDP and PLAG treatment, changes in body weight and feed rate were measured twice a week. The change in the feed rate is calculated by placing a fixed amount of solid food into each cage and checking the weight of the remaining solid food at a specific point in time to calculate the amount of food eaten by the individual cage.

### Immunohistochemistry (IHC) staining

Mouse tissue specimens were fixed in 10% formaldehyde, embedded in paraffin, and sectioned into 5  $\mu$ m slices. The sections were treated with 3% H<sub>2</sub>O<sub>2</sub> for 10 min to block endogenous peroxidase activity, then blocked with bovine serum albumin. Next, the sections were washed in TBST and incubated with a specific primary antibody overnight at 4 °C. For human CK-18, ab52948 human specific target antibody from Abcam was used, and antibody for myostatin, ab124721 from Abcam (Abcam, Cambridge, UK) was used. Negative controls were incubated with primary normal serum IgG of the species from which the primary antibody was obtained.

### Hematoxylin & eosin (H&E) staining

Mouse tissue specimens were fixed in 10% formaldehyde, embedded in paraffin, and sectioned into 5  $\mu$ m slices. The sections were stained with hematoxylin (Dako, Santa Clara, CA, USA) for 10 min and rinsed in Scott's tap water (2% NaHCO<sub>3</sub> in water). Then, the sections were washed in 100% EtOH and stained with eosin (Dako, Santa Clara, CA, USA) for 5 min. The muscle fiber diameter and distribution rate in muscle tissue was analyzed using Image J.

### Analysis of secreted proteins by ELISA

IL-6 and TNF- $\alpha$  levels secreted in mouse serum were analyzed by factor-specific ELISA (BD bioscience, MN, USA) according to the manufacturer's protocol. Mouse blood was collected through cardiac puncture and stored in EDTA-coated tubes until analysis. To separate the serum for analysis, whole blood was centrifuged at 8800 rpm for 5 min to separate the serum from the aqueous layer. Absorbance was measured at 450 nm using Varioskan LUX (Thermoscientific, Waltham, MA, USA). Then, after drawing a quantitative curve using the standard provided by the manufacturer, protein levels were quantified by substituting the absorbance measured in each sample.

### Glucose consumption quantitation assay

The total amount of glucose consumption by muscle tissue was quantitatively analyzed using a glucose uptake kit (Abcam, Cambridge,

UK) according to the manufacturer's protocol. Absorbance was measured at 450 nm using Varioskan LUX (ThermoScientific, Waltham, MA, USA). After drawing a quantitative curve using the standard provided by the manufacturer, glucose consumption was quantified by substituting the absorbance measured in each sample.

### Size of muscle fiber analysis

Muscle fiber size was quantified using Muscle J from Image J. The original three muscle tissue H&E stains for each test group were entered into the Muscle J program. Muscle J quantitatively analyzes the size of each muscle fiber by considering the microscopic ratio in each original H&E picture and derives the final number by dividing it by size.

### Statistics

The data were analyzed using Prism 9 (GraphPad Software, La Jolla, CA, USA).  $p < 0.05$  was considered statistically significant.

## Results

### Severe cisplatin-induced cachexia symptoms were effectively alleviated in PLAG-treated mice

Tumor cells were orthotopically implanted in mice, and these mice were treated with cisplatin to reduce tumor burden, which led to severe cachexia. The degree of cachexia was evaluated by examining muscle contraction, diet, and weight loss. Because severe cachexia symptoms are the most frightening side effects that prevent optimal tumor treatment and delay recovery, mice were treated with PLAG therapy to alleviate cisplatin-induced cachexia symptoms. Mice administered cisplatin for 6 weeks appeared weak, exhibited weight loss, and had severe muscle contraction. On the other hand, mice co-treated with PLAG exhibited a phenotype similar to normal mice (Fig. 1A,B). These results suggest PLAG promotes rapid recovery of cisplatin-induced cachexia symptoms. This cachexia mitigation effect was verified by monitoring body weight and food intake twice a week for 6 weeks. In

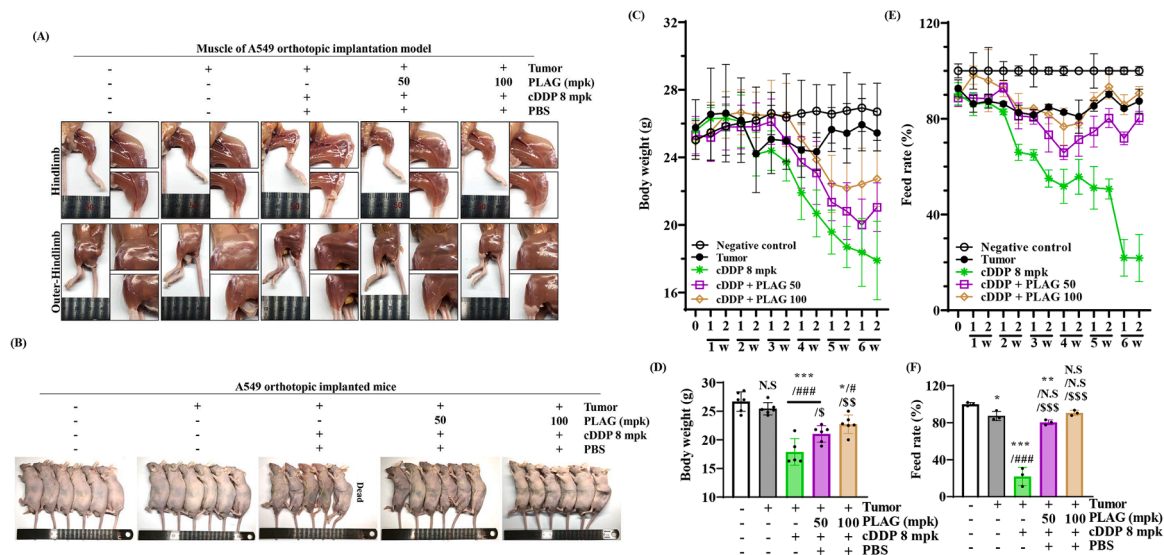
cisplatin-treated mice, body weight and food intake began to decrease after 2 weeks of treatment. After 6 weeks of cisplatin treatment, body weight decreased by about 30% and food intake decreased by about 80%. On the contrary, body weight improved to normal levels in a concentration-dependent manner in the PLAG co-treatment group after the sixth week. In particular, the feed intake began to recover 4 weeks after treatment and returned to a completely normal level 6 weeks after PLAG co-treatment (Fig. 1C-F).

### No severe cachexia symptoms were found in tumor-implanted mice

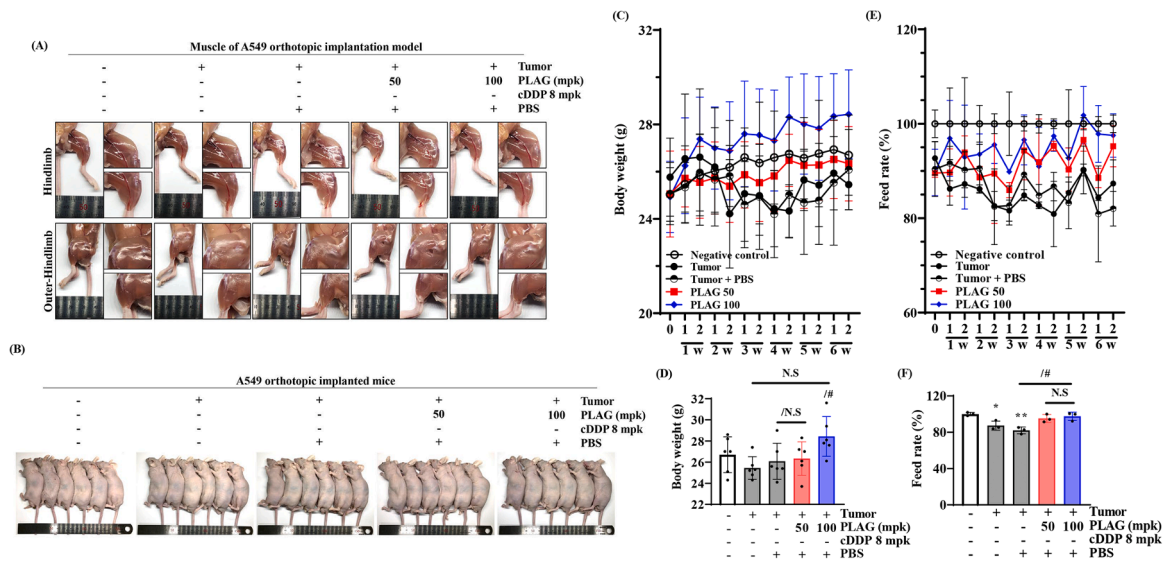
Massive tumor burden can sometime induce cachexia symptoms [31, 32]. A549 cells were implanted orthotopically, which resulted in cancer burden within lung tissue after 6 weeks. During tumor development in the absence of cisplatin therapy, severe cachexia symptoms were not observed in the external phenotype of mice and femur muscles. No specific phenotype was observed in the PLAG co-treated group (Fig. 2A, B). Weight was measured twice a week for 6 weeks in tumor-bearing mice. Slight weight loss (not significant) was observed in tumor-bearing mice, and this slight weight loss recovered in PLAG co-treatment mice. Mice were sacrificed and weighed 6 weeks after tumor implantation. Tumor-implanted mice exhibited a slight weight loss, and recovery from weight loss in PLAG co-treated mice was concentration-dependent (Fig. 2C,D). Additionally, food intake was measured twice a week for 6 weeks. Food intake was slightly reduced in tumor-bearing mice, and intake was restored six weeks after tumor implantation in PLAG co-treated mice (Fig. 2E,F).

### Severe cachexia was evaluated by quadriceps femoris and gastrocnemius weight

Muscle atrophy is one of the major symptoms associated with cisplatin-induced cachexia. Large muscles, such as the quadriceps and gastrocnemius, need to contract for general activities. Therefore, muscle weight reduction is the most important cause of decreased activity and quality of life in cancer patients. Quadriceps and gastrocnemius were isolated and quantified after 6 weeks of treatment with either cisplatin



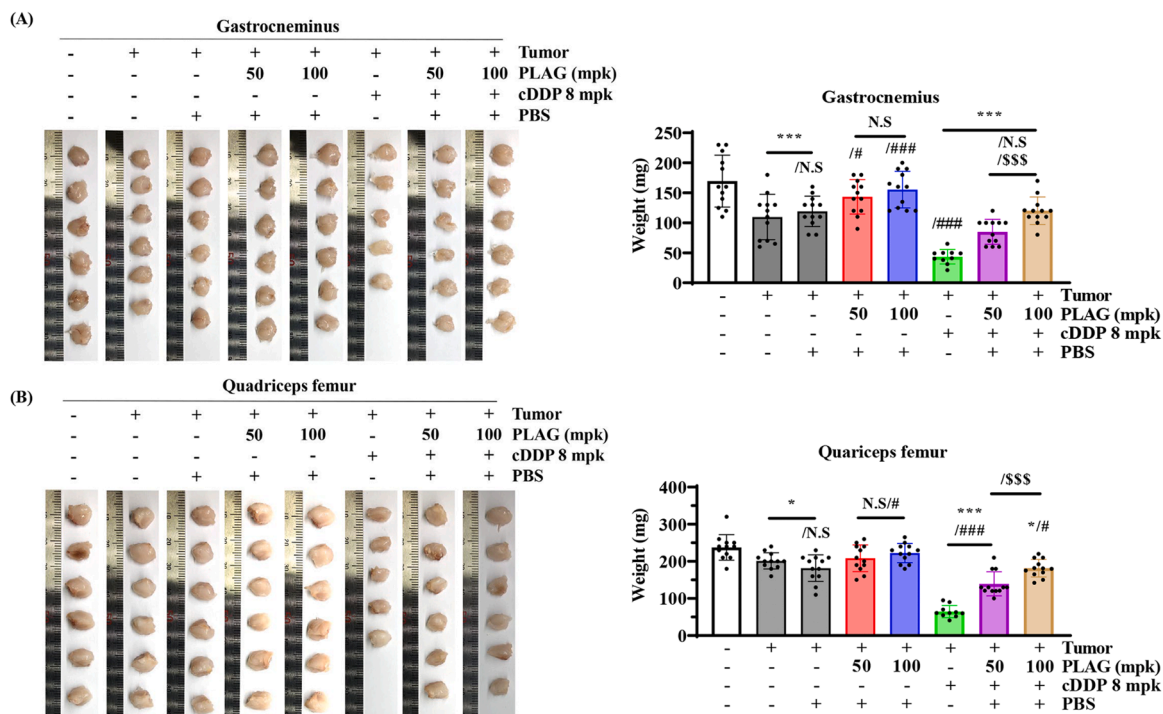
**Fig. 1.** PLAG effectively relieves cisplatin-induced cachexia symptoms. (A) Changed morphology and reduced major leg muscle size were examined in cisplatin and/or PLAG-treated mice on the sacrifice day. (B) Morphology of cachexia symptoms induced by cisplatin and phenotypically recovered mice in PLAG co-treated mice. (C) Weight loss induced by cisplatin and weight recovery following PLAG treatment were confirmed twice a week for 6 weeks. (D) After 6 weeks, weight differences between each experimental group were compared. (E) Reduced food intake by cisplatin and recovery of food intake by PLAG treatment were confirmed twice a week for 6 weeks. (F) After 6 weeks, the difference in food intake between each experimental group was compared. Compared to normal mice: \* $p < 0.033$ , \*\* $p < 0.002$ , \*\*\* $p < 0.001$ ; Compared to the tumor only: # $p < 0.033$ , ## $p < 0.002$ , ### $p < 0.001$ , Compared to the cisplatin only: \$ $p < 0.033$ , \$\$ $p < 0.002$ , \$\$\$ $p < 0.001$  (each experiment  $n = 12$  and  $n = 10$  for cisplatin only). N.S, not significant. Mean  $\pm$  SD.



**Fig. 2.** PLAG relieves symptoms of mild cachexia caused by tumor growth (A) Changed morphology and reduced major leg muscle size were examined in A549 cell-implanted mice on the sacrifice day. (B) Morphology of cachexia symptoms induced in A549 cell-implanted mice and phenotypically recovered mice in PLAG co-treated mice. (C) Weight loss in A549 cell-implanted mice and weight recovery by PLAG treatment were confirmed twice a week for 6 weeks. (D) After 6 weeks, weight differences between each experimental group were compared. (E) Reduced food intake in A549 cell-implanted mice and recovery of food intake by PLAG treatment were confirmed twice a week for 6 weeks. (F) After 6 weeks, the difference in food intake between each experimental group was compared. Compared to normal mice: \* $p < 0.033$ , \*\* $p < 0.002$ , \*\*\* $p < 0.001$ ; Compared to the tumor only: # $p < 0.033$ , ## $p < 0.002$ , ### $p < 0.001$ , Compared to the cisplatin only: \$ $p < 0.033$ , \$\$ $p < 0.002$ , \$\$\$ $p < 0.001$  (each experiment  $n = 12$ ). N.S, not significant. Mean  $\pm$  SD.

or cisplatin and PLAG. In cisplatin-treated mice, the weight of the quadriceps and gastrocnemius decreased by about 30% compared to normal mice. In the PLAG co-treatment group, these two large femur muscles recovered to almost normal levels, depending on the treatment concentration. Specifically, the gastrocnemius recovered to a level

similar to that of normal mice in the group co-treated with PLAG at 100 mg/kg (Fig. 3A,B).



**Fig. 3.** Femur muscle reduction due to cisplatin treatment and their recovery through PLAG treatment. (A) Gastrocnemius, one of major leg muscles, was isolated and size and weight in cisplatin-treated mice were evaluated and compared between each experimental group on the sacrifice day. (B) Quadriceps, one of major leg muscle, was isolated and size and weight in cisplatin-treated mice were evaluated and compared between each experimental group on the sacrifice day. Compared to normal mice: \* $p < 0.033$ , \*\* $p < 0.002$ , \*\*\* $p < 0.001$ ; Compared to the tumor only: # $p < 0.033$ , ## $p < 0.002$ , ### $p < 0.001$ , Compared to the cisplatin only: \$ $p < 0.033$ , \$\$ $p < 0.002$ , \$\$\$ $p < 0.001$  (each experiment  $n = 12$  and  $n = 10$  for cisplatin only). N.S, not significant. Mean  $\pm$  SD.

### PLAG effectively restores cisplatin-damaged muscle fibers

Muscle loss due to cisplatin resulted in muscle fiber atrophy. Gastrocnemius and quadriceps muscle fiber wasting was analyzed through H&E tissue staining and IHC staining using myostatin antibody. Wasting muscle fibers can be identified by their reduced overall size. Cisplatin treatment reduces the size of muscle fibers in the gastrocnemius and quadriceps. Muscle fibers of 700 to 1000  $\mu\text{m}^2$  in normal mice atrophied to less than 500  $\mu\text{m}^2$  in cisplatin-treated mice. However, PLAG co-treatment restored muscle fibers to their normal size. In particular, 100 mg/kg PLAG co-treatment restored the size of muscle fibers to the same level as normal mice. Also, the mean diameter of each muscle was effectively recovered in PLAG co-treated mice. Furthermore, high myostatin expression, a known cachexia marker, which was observed in muscles of cisplatin-treated mice was reduced to normal levels in the PLAG co-treatment group along with muscle fiber recovery (Fig. 4A,B).

### PLAG normalizes the major factors modified in mice with cisplatin-induced cachexia

Cachexia may be induced through the excessive expression of inflammation-related factors, which are upregulated due to muscle atrophy. IL-6 and TNF- $\alpha$  are inflammatory factors that have been associated with cachexia. These two factors induce muscle fiber reduction and cause muscle wasting through activation of intra-muscle signaling pathways, which eventually inhibit glucose metabolism in muscle cells. In mice that were implanted with human tumor cells, human interleukin-6 (hIL-6) increased by about 2.5–3 times and returned to normal levels following 100 mg/kg of PLAG treatment. In mice treated with cisplatin, hIL-6 was slightly increased. These data indicated enhanced hIL-6 expression may be the result of growing implanted human cells rather than cisplatin therapy. Meanwhile, human TNF- $\alpha$  (hTNF- $\alpha$ ) increased slightly in human tumor cell-implanted mice but substantially increased in cisplatin-treated mice. These results demonstrated that increased hTNF- $\alpha$  expression occurs primarily in response to cisplatin therapy rather than growth of implanted human cells (Fig. 5A). Taken together, this data suggests implanted tumor cells express hIL-6 during tumor growth, but cisplatin therapy stimulates hTNF- $\alpha$  expression. In PLAG co-treated therapy, a return to normal hIL-6 levels

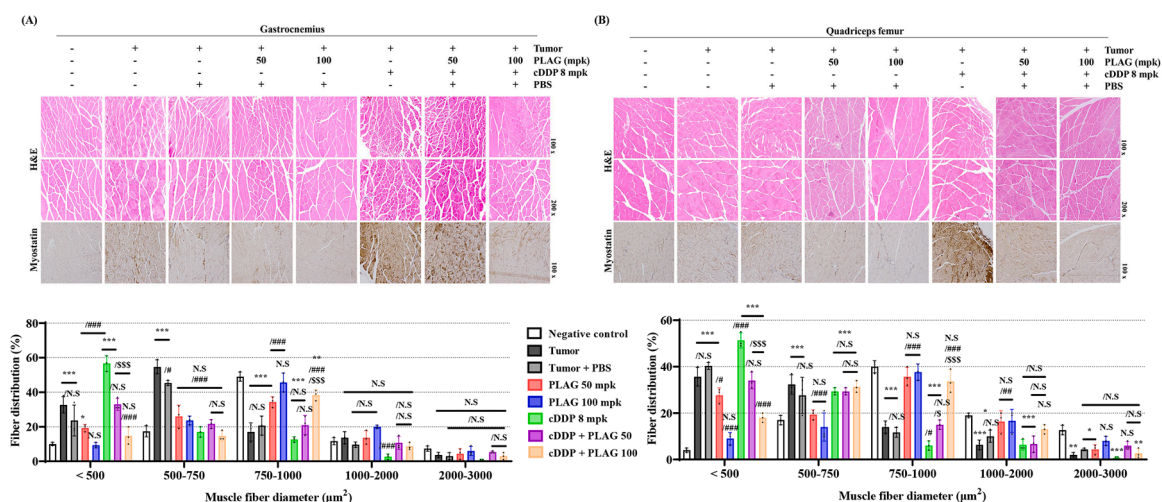
indicates tumor growth inhibition, whereas normalized TNF- $\alpha$  expression indicates reduced inflammation. In addition, mouse IL-6 (mIL-6) and mouse TNF- $\alpha$  (mTNF- $\alpha$ ) expression in tumor-bearing cisplatin-treated mice was produced in mouse cells that form the tumor stroma. Cisplatin treatment induced a 5-fold increase in mIL-6 and 12-fold increase in mTNF- $\alpha$  expression, and these levels were reduced to normal through PLAG therapy. In particular, in the group treated with 100 mg/kg PLAG, mIL-6 was reduced to the same level as normal mice (Fig. 5B). In addition to the increase in cytokines associated with cachexia symptoms, glucose absorption in the two large leg muscles, gastrocnemius and quadriceps, decreased significantly in the cisplatin treatment group but recovered to almost normal levels in the PLAG co-treated group (Fig. 5C).

### PLAG does not significantly affect the antitumor effect of cisplatin but alleviates cisplatin-induced cachexia symptoms

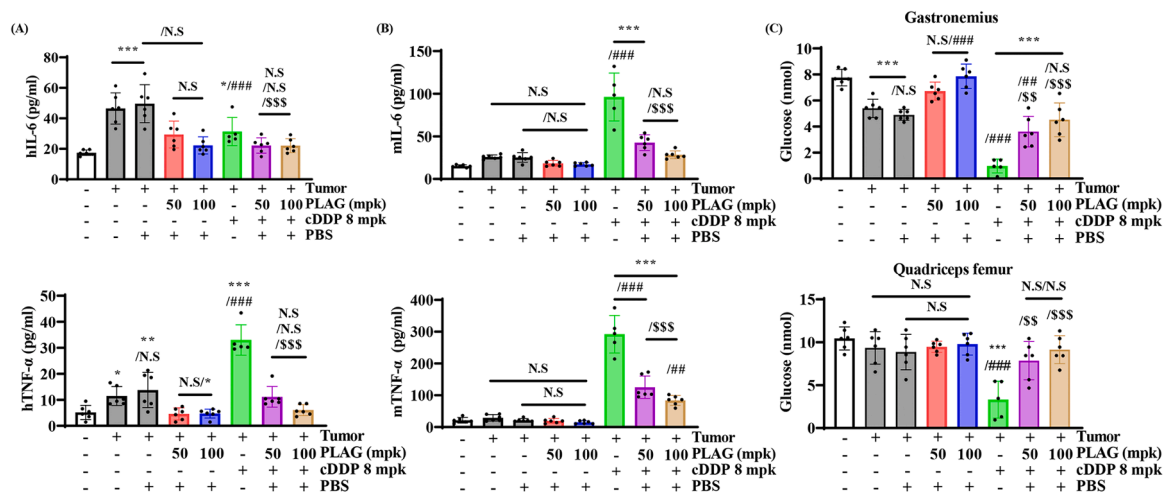
Cisplatin chemotherapy is generally administered as the standard of care therapeutics to treat cancer patients. Therefore, the best therapy to alleviate cachexia symptoms must be combined with therapy that mediates complete removal of tumor. To examine delay in tumor formation, A549 cells were implanted orthotopically into the lungs. Then, the mice were treated with cisplatin, PLAG, and PLAG/cisplatin to mitigate cachexia symptoms and tumor burden was examined with H&E staining and IHC staining using anti-hCK18. H&E and IHC stained lung showed that alveolar tissue was filled with aggressive growing tumor. No aggressive growth tumor cells were observed in cisplatin, PLAG, and PLAG/cisplatin co-treatment mice (Fig. 6A). The lung tissue weight in A549 cell-implanted mice increased slightly and returned to normal levels following PLAG, cisplatin, and PLAG/cisplatin treatment (Fig. 6B). These data indicate PLAG alleviated cachexia symptoms, had a practical anti-tumor effect, and did not interfere with the effects of cisplatin. Our data demonstrates that PLAG is a vital substance that can effectively relieve cachexia symptoms without inhibiting the anti-tumor effect of cisplatin.

## Discussion

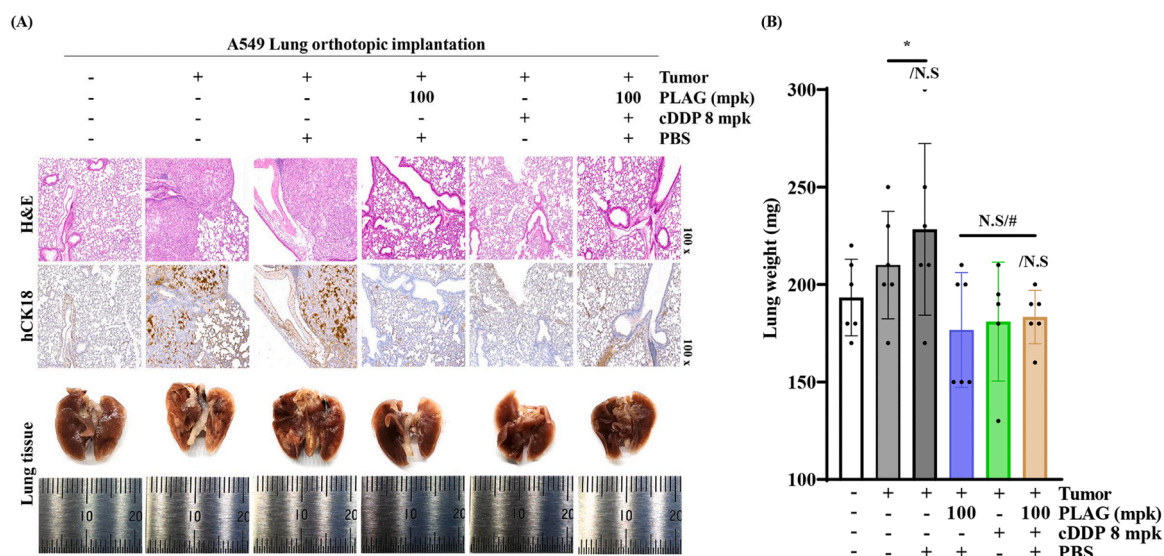
Chemotherapy remains an important and indispensable treatment



**Fig. 4.** PLAG restores muscle fiber size distribution, which is reduced by cisplatin. (A) In cisplatin-treated mice, muscle fiber size reduction in gastrocnemius muscle and restoration of muscle fibers with PLAG combination therapy were evaluated through H&E staining. Increased levels of myostatin expression in gastrocnemius muscle, which were induced by cisplatin and recovered by PLAG, were evaluated through IHC staining with anti-myostatin (B) The reduction in muscle fiber size of the quadriceps femoris following cisplatin treatment and the restoration of muscle fibers by adding PLAG were evaluated through H&E staining. Increased levels of myostatin expression induced by cisplatin in the quadriceps femoris, and recovery by PLAG were evaluated by IHC staining with anti-myostatin. Compared to normal mice: \* $p < 0.033$ , \*\* $p < 0.002$ , \*\*\* $p < 0.001$ ; Compared to the tumor only: # $p < 0.033$ , ## $p < 0.002$ , ### $p < 0.001$ , Compared to the cisplatin only: \$ $p < 0.033$ , \$\$ $p < 0.002$ , \$\$\$ $p < 0.001$  (each experiment  $n = 6$ ). N.S, not significant. Mean  $\pm$  SD.



**Fig. 5.** Normalization of increased cachexia-related factors by PLAG treatment (A) Increase of human interleukin-6 (hIL-6) and human TNF- $\alpha$  (hTNF- $\alpha$ ) in implanted human lung cancer cells following cisplatin therapy and normalization of cytokine levels by PLAG addition were quantified by ELISA analysis. (B) Increase of mouse interleukin-6 (mIL-6) and mouse TNF- $\alpha$  (mTNF- $\alpha$ ) by cisplatin therapy and normalization of cytokine levels by PLAG addition were quantified by ELISA analysis. (C) Glucose absorption in the gastrocnemius and quadriceps was evaluated using a glucose consumption assay, and the difference in glucose absorption between each experimental group was compared to normal mice: \* $p < 0.033$ , \*\* $p < 0.002$ , \*\*\* $p < 0.001$ ; Compared to the tumor only: # $p < 0.033$ , ## $p < 0.002$ , ### $p < 0.001$ , Compared to the cisplatin only: \$ $p < 0.033$ , \$\$ $p < 0.002$ , \$\$\$ $p < 0.001$  (each experiment  $n = 12$  and  $n = 10$  for cisplatin only). N.S, not significant. Mean  $\pm$  SD.



**Fig. 6.** PLAG has an anti-tumor effect and does not interfere with the anti-tumor effect of cisplatin. (A) The alveolar tissue filled with implanted A549 NSCLC was examined with H&E staining and tumor cells were identified through immunochemical staining with hCK-18 (human specific tumor target protein). (B) The weight of the lungs, including implanted tumor cells was evaluated, and weight differences were compared between each experimental group. Compared to normal mice: \* $p < 0.033$ , \*\* $p < 0.002$ , \*\*\* $p < 0.001$ ; Compared to the tumor only: # $p < 0.033$ , ## $p < 0.002$ , ### $p < 0.001$ , Compared to the cisplatin only: \$ $p < 0.033$ , \$\$ $p < 0.002$ , \$\$\$ $p < 0.001$  (each experiment  $n = 12$  and  $n = 10$  for cisplatin only). N.S, not significant. Mean  $\pm$  SD.

for cancer patients. Various cancer therapeutics have been developed, but more efficacy studies are needed to develop practical anti-cancer drugs beyond chemotherapy [33, 34]. Despite the anti-cancer effects of chemotherapy, severe side effects limit its practical and extensive use. In this paper, we propose a combination treatment containing cisplatin that can increase the efficacy of anti-cancer drugs and reduce its side effects. Though cisplatin effectively reduces tumor burden, it often causes cachexia symptoms. In fact, typical cachexia symptoms, such as loss of food intake and rapid weight loss, were observed in mice treated with 8 mg/kg cisplatin weekly. PLAG and cisplatin co-treatment effectively alleviated cachexia symptoms. In mice treated with 100 mg/kg PLAG, decreased food intake and weight loss, induced by cisplatin therapy, were restored to 100% and 80% levels of normal mice,

respectively (Fig. 1)

Severe cachexia symptoms make it difficult for patients to exercise daily. Protection of femur muscles, including quadriceps and gastrocnemius, is essential to maintain the daily life of cancer patients suffering from cachexia symptoms [35–37]. In cisplatin-treated mice, the size and weight of the two major leg muscles decreased to 70% but recovered with PLAG combination therapy. In the group treated with 100 mg/kg PLAG, decreased femur muscles by cisplatin recovered up to about 85% compared to normal mice (Fig. 3). Chemotherapy-induced muscle wasting begins with the atrophy of muscle fibers into small units, which is due to the expression of factors that induce muscle wasting, such as myostatin [17, 38, 39]. Muscle fibers atrophied to less than 500  $\mu\text{m}^2$  following cisplatin treatment and were effectively restored to 90% of

normal levels in PLAG-treated mice. In addition, increased myostatin expression in muscle fibers following cisplatin therapy was reduced to normal levels in PLAG co-treated mice (Fig. 4).

Severe and chronic inflammation has been known to contribute to cachexia symptoms. Inflammatory factors such as IL-6 and TNF- $\alpha$  generally increase during chemotherapy. These cytokines eventually lead to muscle atrophy through increased muscle damage-related factors, such as myostatin and Muscle RING-finger protein-1 [40–44]. IL-6 and TNF- $\alpha$ , which were rapidly increased by cisplatin treatment, were reduced to the same level as negative controls in mice co-treated with 100 mg/kg PLAG and cisplatin. Glucose intake of muscle cells was tested to evaluate the regular activity of muscle tissue [39, 45–48]. Cisplatin lowered glucose absorption in both major leg muscles to less than 10% of normal mice. PLAG restored glucose absorption to a level similar to that of normal mice (Fig. 5).

Receiving adequate chemotherapy to completely remove tumors requires more extended therapeutic periods, but continuous treatment is difficult due to the severe side effects associated with chemotherapy, including cachexia. Therefore, it is essential to develop a combined therapy that relieves cachexia symptoms so cancer patients can receive prolonged chemotherapy to ensure complete tumor removal. For this purpose, we developed a candidate substance that could alleviate cachexia symptoms without significantly affecting the antitumor effect of chemotherapy. PLAG is present in Sika deer antlers and can be synthesized from glycerol, palmitic acid, and linoleic acid. Our previous study demonstrated that PLAG effectively alleviated the symptoms of oral mucosal inflammation caused by chemotherapy [24, 25]. In addition, PLAG effectively alleviated symptoms of various inflammatory diseases induced by several factors [26–30]. In these papers, PLAG effectively regulated intracellular ROS, which prevented tissue damage caused by specific factors and suppressed severe inflammatory responses. Cisplatin-induced cachexia symptoms are recognized as the cause of muscle tissue damage due to excessive ROS. Cisplatin induces continuous ROS generation in muscle tissue, resulting in damage to muscle tissue and muscle atrophy [49–51]. However, PLAG effectively regulates ROS in these tissues and reduces muscle damage in cisplatin therapy. Taken together, PLAG represents a potential drug that can relieve chemotherapy-induced cachexia symptoms in cancer patients.

#### CRedit authorship contribution statement

**Guen Tae Kim:** Writing – original draft, Methodology, Validation, Investigation, Formal analysis, Data curation. **Eun Young Kim:** Investigation, Formal analysis. **Su-Hyun Shin:** Investigation. **Hyowon Lee:** Investigation. **Se Hee Lee:** Investigation. **Kaapjoo Park:** Resources, Validation, Writing – review & editing. **Ki-Young Sohn:** Funding acquisition. **Sun Young Yoon:** Conceptualization, Validation. **Jae Wha Kim:** Project administration, Supervision, Conceptualization, Writing – review & editing.

#### Declaration of Competing Interest

The authors declare no potential conflicts of interest

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#### Supplementary materials

Supplementary material associated with this article can be found, in

the online version, at doi:10.1016/j.tranon.2022.101398.

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