


Evaluation of the Efficacy and Safety of the Herbal Formula PM014 in a Cisplatin- and Paclitaxel-Treated Tumor-Bearing Mouse Model

Integrative Cancer Therapies
Volume 19: 1–10
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1534735420924711
journals.sagepub.com/home/ict


Chanju Lee, PhD¹, Hyunju Jeong, MS¹, Kwang Hyung Lee, KMD¹, Sehyun Park, KMD¹, Mi Jung Gang, KMD¹, Soo Kyung Bae, PhD², and Hyunsu Bae, KMD, PhD¹ 

Abstract

PM014 (HL301) is a standardized herbal mixture derived from a traditional Korean medicine, Chung-Sang-Bo-Ha-Tang. Previously, we reported that PM014 treatment significantly suppressed pulmonary fibrosis, one of the frequent adverse effects of anticancer therapy in lung cancer. Before the clinical application of PM014 in anticancer therapy, the safety and efficacy of PM014 in combination with conventional anticancer drugs should be addressed to determine whether PM014 can be used in lung cancer. Lewis lung cancer-bearing mice were injected with 10 mg/kg of cisplatin or paclitaxel on day 5. Starting on day 7, the mice were administered 200 mg/kg PM014 every 2 days. On day 15, all mice were assessed by biochemical and histological analyses. PM014 did not block the antitumor activity of cisplatin and paclitaxel. Coadministration of PM014 and antitumor agents did not elevate the aspartate transaminase/alanine transaminase ratio or the blood urea nitrogen/creatinine ratio. Histopathological analysis also showed that PM014 did not induce hepatic or renal injury. Moreover, PM014 had no apparent inhibitory effects on drug metabolizing enzymes, indicating that PM014 did not alter the pharmacokinetics of chemotherapeutic drugs. Overall, these data show the safety and compatibility of combination therapy of PM014 and chemotherapies for the treatment of lung cancer.

Keywords

PM014, cisplatin, paclitaxel, coadministration, lung cancer, herb drug interaction

Submitted December 20, 2019; revised March 24, 2020; accepted April 16, 2020

Introduction

Lung cancer remains the most common cause of cancer-related death worldwide with a high incidence in both sexes in the last few decades.^{1,2} Most cases of late-stage or locally advanced lung cancer are surgically unresectable, and chemotherapy, radiotherapy, or both are usually considered for these patients.³

Radiotherapy and chemotherapy can successfully remove the tumor cells in most cases but can also lead to a wide range of side effects due to toxicity to normal tissues.⁴ Similar to radiotherapy, a variety of chemotherapeutic agents including bleomycin, gemcitabine, cisplatin (cis), paclitaxel (PTX), docetaxel, and oxaliplatin have been clinically reported to generate pulmonary complications such as infiltrates, interstitial pneumonitis, organizing pneumonitis, pulmonary edema, respiratory failure associated with alveolar

damage, hypersensitivity, and fibrosis.^{5–8} Furthermore, a single chemotherapeutic agent can induce multiple injury patterns, which may progress into fibrosis.^{7,9,10} Pulmonary fibrosis induced by anticancer therapy results in poor clinical outcome by limiting cancer treatments.^{11–14} Therefore, to facilitate an efficient anticancer therapy accompanied with prolonged survival, pulmonary inflammation must be inhibited with proper combination therapy.

¹Kyung Hee University, Seoul, Republic of Korea

²The Catholic University of Korea, Bucheon, Republic of Korea

Corresponding Author:

Hyunsu Bae, Department of Physiology, College of Korean Medicine, Kyung Hee University, 26-6 Kyunghedae-ro, Dongdaemungu, Seoul 02453, Republic of Korea.
Email: hbae@khu.ac.kr



We previously reported that PM014, the 7 major herbal components of Chung-Sang-Bo-Ha-Tang, which has been used in Korea for centuries to treat a variety of pulmonary diseases,¹⁵ inhibits inflammation in acute lung inflammation and chronic obstructive pulmonary disease in mice.¹⁶⁻¹⁸ We recently reported that PM014 treatment recovered both the radiation-induced pulmonary damage and bleomycin-induced epithelial mesenchymal transition in a mice model. Additionally, we reported that 200 mg/kg of PM014 successfully lowered the level of transforming growth factor β -1, a critical profibrotic factor in fibrosis, by inhibiting inflammatory cell infiltration and resulting in reduced pulmonary fibrosis in radiation and chemotherapeutic model.^{19,20} Given that lung cancer with pulmonary fibrosis markedly reduces the survival of patients and radiotherapy or surgical treatment are also ineffective,²¹ a novel strategy of anticancer and antifibrosis drug administration is required for effective and safe treatment. Thus, we designed the experiment to verify the possibility of combination therapy of PM014 and standard chemotherapies and examined whether PM014 can be used with cis or PTX safely without interfering the antitumor activity in a lung cancer mouse model.

Materials and Methods

Cell Culture

The murine Lewis lung carcinoma (LLC) cell line (kindly provided by Dr Sung-Hoon Kim, Kyung Hee University, Korea) was maintained in Dulbecco's modified Eagle's medium (DMEM; Welgene) supplemented with 10% heat-inactivated fetal bovine serum (Welgene), 100 U/mL penicillin, and 100 μ g/mL streptomycin (Invitrogen). The cells were cultured every 2 to 3 days until reaching 80% confluence.

Animal Experiment

Male C57BL/6 (6 weeks old, 20-22 g) wild-type mice were purchased from DBL and randomly divided into 4 groups as follows: set I: control (n = 5), PM014 (n = 5), cis (n = 6), and cis + PM014 (n = 6); and set II: control (n = 6), PM014 (n = 6), PTX (n = 6), and PTX + PM014 (n = 6); average weight was approximately 21 g. The mice were put in cages of maximum 5 mice each.

LLC cells were mixed with Matrigel matrix (Corning) and inoculated subcutaneously in the right flank (5×10^4 cells/mouse). Cis or PTX was injected once at day 5 after tumor challenge. The mice were injected with 200 mg/kg PM014 every 2 days for a total of 5 times, starting 2 days after the cis or PTX injection. All tumor tissues were harvested at day 15 after tumor inoculation, and images were captured digitally using a SONY NEX-5 digital camera (Sony Corp).

All animals were maintained in a specific pathogen-free environment on a 12-hour light/dark cycle with free access to food and water. Nesting sheets were used for enrichment. After the termination of experiments, blood was drawn by cardiac puncture under 2% isoflurane anesthesia and all mice were euthanized by isoflurane and cervical dislocation. The animal studies were approved by the University of Kyung Hee Institutional Animal Care and Use of Committee (KHUASP(SE)-18-064).

Chemicals

Cisplatin and PTX were obtained from Sigma-Aldrich. Cis was dissolved in normal saline at 1 mg/mL, and PTX was dissolved in 1:1 = ethanol:kolliphor EL solution at 5 mg/mL. All solutions were stored in the dark and diluted with normal saline.

The herbal formula of PM014 was obtained from Kyung Hee Herb Pharm. The detailed protocol was described in a previous study.¹⁹ Briefly, the 7 herbs (root of *Rehmannia glutinosa*: cortex of *Paeonia suffruticosa*: fruit of *Schizandra chinensis*: root of *Asparagus cochinchinensis*: seed of *Prunus armeniaca*: root of *Scutellaria baicalensis*: root of *Stemona sessilifolia* in the ratio 4:2:2:2:1.5:1.5:1) were extracted in purified water and evaporated. The extracts named PM014 were vacuum dried. PM014 was dissolved in phosphate-buffered saline for administration. The standardized herbal formula of PM014 (HL301) has been approved for the Investigational New Drug program by the Ministry of Food and Drug Safety, Republic of Korea (20130030575), and registered at www.clinicaltrials.gov (NCT03309800 and NCT03654196).

Pooled human liver microsomes from 150 donors (75 males and 75 females) were purchased from Corning Life Sciences. β -Nicotinamide adenine dinucleotide phosphate disodium salt, glucose 6-phosphate disodium salt hydrate, glucose 6-phosphate dehydrogenase, $MgCl_2$, and all chemicals including the specific substrates, its metabolites, and well-known inhibitors of 9 cytochrome P450s (CYPs) were purchased from Sigma-Aldrich Corporation, Santa Cruz Biotechnology, or Cayman Chemicals unless stated otherwise.

Inhibitory Effects of PM014 Toward the 9 CYP Isoforms in Human Liver Microsomes Through Cocktail Assays

The inhibitory effects of PM014 on CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 were evaluated in pooled human liver microsomes through the use of specific CYP probe substrates (cocktail assay), as previously described²² with a slight modification. Combining 9 specific substrates for each

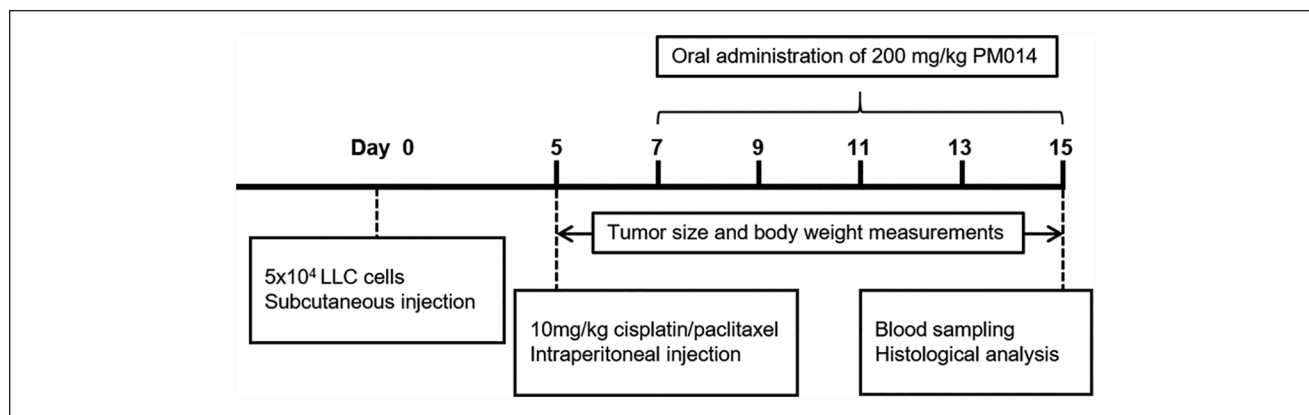


Figure 1. The experimental schedule of coadministration of cisplatin or paclitaxel and PM014. Cisplatin or paclitaxel (10 mg/kg for both) was administered 5 days after tumor inoculation via intraperitoneal injection. Two days after the initial injection, 200 mg/kg of PM014 was orally injected every 2 days, for a total of 5 injections.

CYP cocktails are particularly useful because it is possible to measure multiple CYP activities in the same microsomal incubation. In brief, the 90- μ L incubation mixture, including pooled human liver microsomes (final concentration 0.1 mg/mL), 0.1 M phosphate buffer (pH 7.4), each P450-selective substrate cocktail set, and PM014 methanol extract (0-200 μ g/mL) was preincubated for 5 minutes at 37 °C.

The reaction was initiated by adding a 1.3 mM NADPH-generating system and was then incubated for 15 minutes at 37 °C in a shaking water bath. After incubation, reactions were stopped by the addition of 200- μ L ice-cold acetonitrile containing 2 μ M chlorpropamide as an internal standard, and then they were centrifuged (13 000 rpm for 8 minutes at 4 °C). The 5 μ L of the supernatant was injected into the liquid chromatography–tandem mass spectrometry system. All incubations were performed in triplicate, and mean values were used for analysis. To determine the magnitude of inhibitory effects, we included identical parallel incubation samples containing well-known reversible CYP inhibitors for each CYP isozyme, all of which appear on the US Food and Drug Administration list of recommended or accepted *in vitro* inhibitors.

Blood Collection and Enzyme Assays

Blood was drawn by cardiac puncture from the heart. The blood was incubated at room temperature for clotting for 3 hours. The blood was centrifuged (3000 rpm for 30 minutes at 4 °C), and the supernatant was collected for the estimation of hepatic and urinary enzymes to assess the hepatotoxicity and nephrotoxicity. The enzymes were measured at Genia Inc.

Histopathology

Liver and kidney were fixed overnight with paraformaldehyde, dehydrated, and embedded in paraffin. The tissues

were cut on a rotary microtome at 4 μ m thickness. After deparaffinization and rehydration, the sections were stained with hematoxylin and eosin. Tissue architecture and inflammatory cell infiltration were observed under the microscope. The histological scoring was performed by counting glomerular damage, inflammatory infiltration, and tubular damage in 10 random spots. Damage was graded as follows: 0% to 9% damage: 0; 10% to 29% damage: 1; 30% to 59% damage: 2; 60% to 79% damage: 3; and \geq 80% damage: 4. Final scores were calculated by averaging all the damage scores for the number of examined mice.

Statistics

All data are expressed as the means and standard errors of the means. Statistical analysis for group comparisons was conducted using 1-way analysis of variance followed by Tukey's post hoc test and comparisons of different time points were conducted using 2-way analysis of variance in Prism 5.01 software (GraphPad Software Inc). *P* value less than .05 was considered significant.

Results

Effect of PM014 on Anticancer Activity of Cisplatin and Paclitaxel *In Vivo*

Previously, we reported that PM014 lowers the lung inflammation induced by radiation. To use PM014 as a drug for pulmonary fibrosis in a lung cancer model, we evaluated whether PM014 would affect the anticancer activity of cis and PTX. Murine LLC tumor-inoculated mice were injected with 10 mg/kg cis/PTX or vehicle (normal saline/ethanol + kholiphor + normal saline). After 2 days, the mice were orally administered 200 mg/kg of PM014 or vehicle (phosphate-buffered saline; Figure 1).

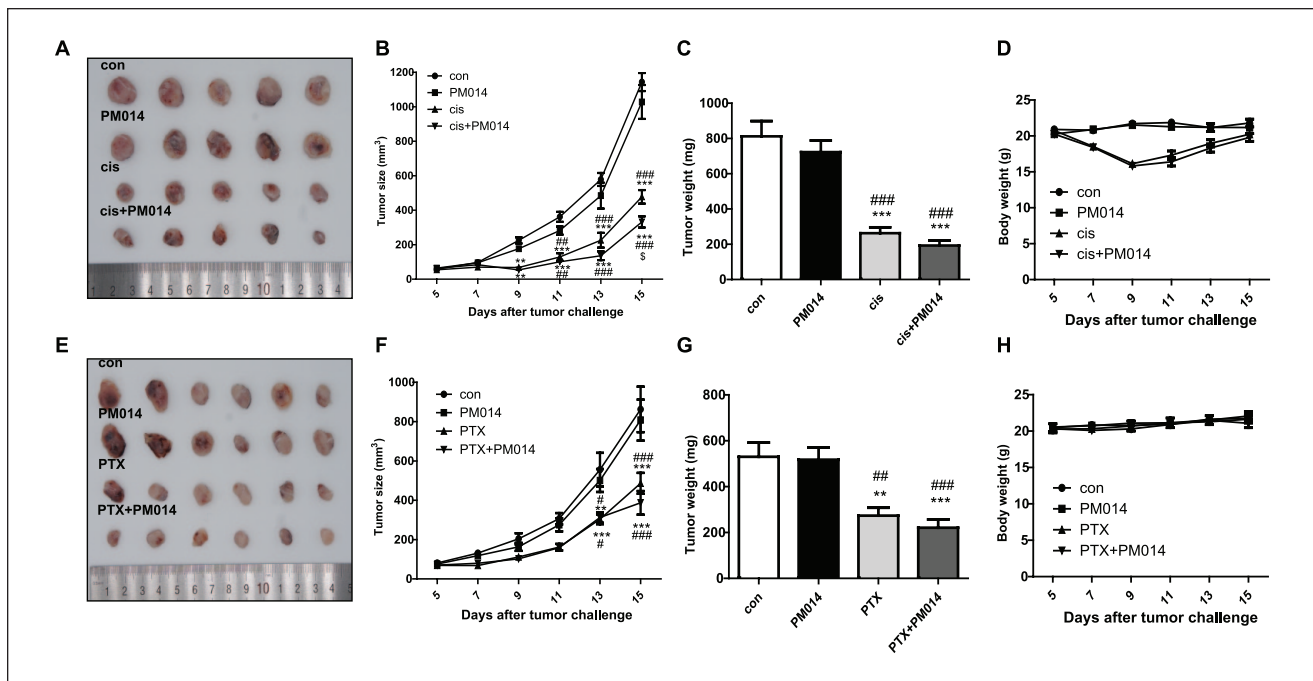


Figure 2. The effect of PM014 on the anticancer activity of cisplatin or paclitaxel in an LLC tumor-bearing mouse model. (A) The representative tumor images of control and PM014 with or without cisplatin injection were obtained on day 15. (B-D) Tumor growth and weight was measured and body weight changes were measured every 2 days. (E) The representative tumor images of control and PM014 with or without paclitaxel injection were obtained to compare the tumor size. (F-G) Tumor and body weight changes were measured. All data are presented as the means \pm SEMs. $**P < .01$, $***P < .001$ versus the control group; $\#P < .05$, $\#\#P < .01$, $\#\#\#P < .001$ versus the PM014 group.

PM014 alone did not provide a significant anti-tumor effect itself compared with the control. Tumor weight showed no difference between the control and PM014. Cis markedly reduced the tumor size and tumor weight compared with the control and PM014. Cis + PM014 coadministration enhanced the anticancer effect of cis and this was statistically significant (Figure 2A-C). Cis-treated mice showed body weight loss after the injection from day 7 to 9 regardless of whether PM014 was administered. Mice with severe body weight loss were euthanized and excluded (cis: 1/6, cis + PM014: 1/6). After day 11, both cis- and cis + PM014-treated mice showed body weight recovery (Figure 2D). Similarly, PTX also reduced the tumor size and tumor weight compared with the control and PM014. Coadministration of PTX + PM014 also showed a significant reduction of tumor growth compared with the control group, but did not show significant difference compared with the PTX group (Figure 2E-G). No body weight loss was observed in any group (Figure 2H). These results showed that PM014 did not block the anticancer effect of cis and PTX in the LLC-bearing mouse model. Furthermore, these results suggested that coadministration of PM014 can enhance the anticancer effect, and that cis shows greater efficacy than PTX when combined with PM014.

The Effect of Coadministration of Cis and PM014 on Hepatotoxicity and Nephrotoxicity

The safety assessment of the drug coadministration was assessed via enzyme measurements in the blood serum of cis- or PTX-treated LLC-bearing mice. The level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was used as a predictor of hepatocellular injury. In the cis model, the vehicle-treated control group showed a normal range of AST and ALT. The mean level of AST and ALT in the control group was 194.83 ± 35.57 IU/L and 50.17 ± 12.31 IU/L, respectively. PM014 (AST = 231.33 ± 32.49 IU/L; ALT = 53.67 ± 9.01 IU/L), cis (AST = 162.70 ± 13.91 IU/L; ALT = 34.43 ± 1.79 IU/L), and cis + PM014 (AST = 184.80 ± 61.80 IU/L; ALT = 42.40 ± 14.14 IU/L) did not show significant changes in either the AST or ALT level (Figure 3A and B). The AST/ALT ratio did not differ between the control and other groups (Figure 3C). Blood urea nitrogen (BUN) and creatinine (CREA) were measured in the serum to verify the effect of PM014 on renal function. The mean level of BUN in the PM014 group compared with control showed no difference (control = 25.27 ± 3.01 mg/dL vs PM014 = 22.63 ± 2.16 mg/dL). Although cis treatment slightly elevated the level of BUN (30.41 ± 1.67 mg/dL in cis), there was no significant

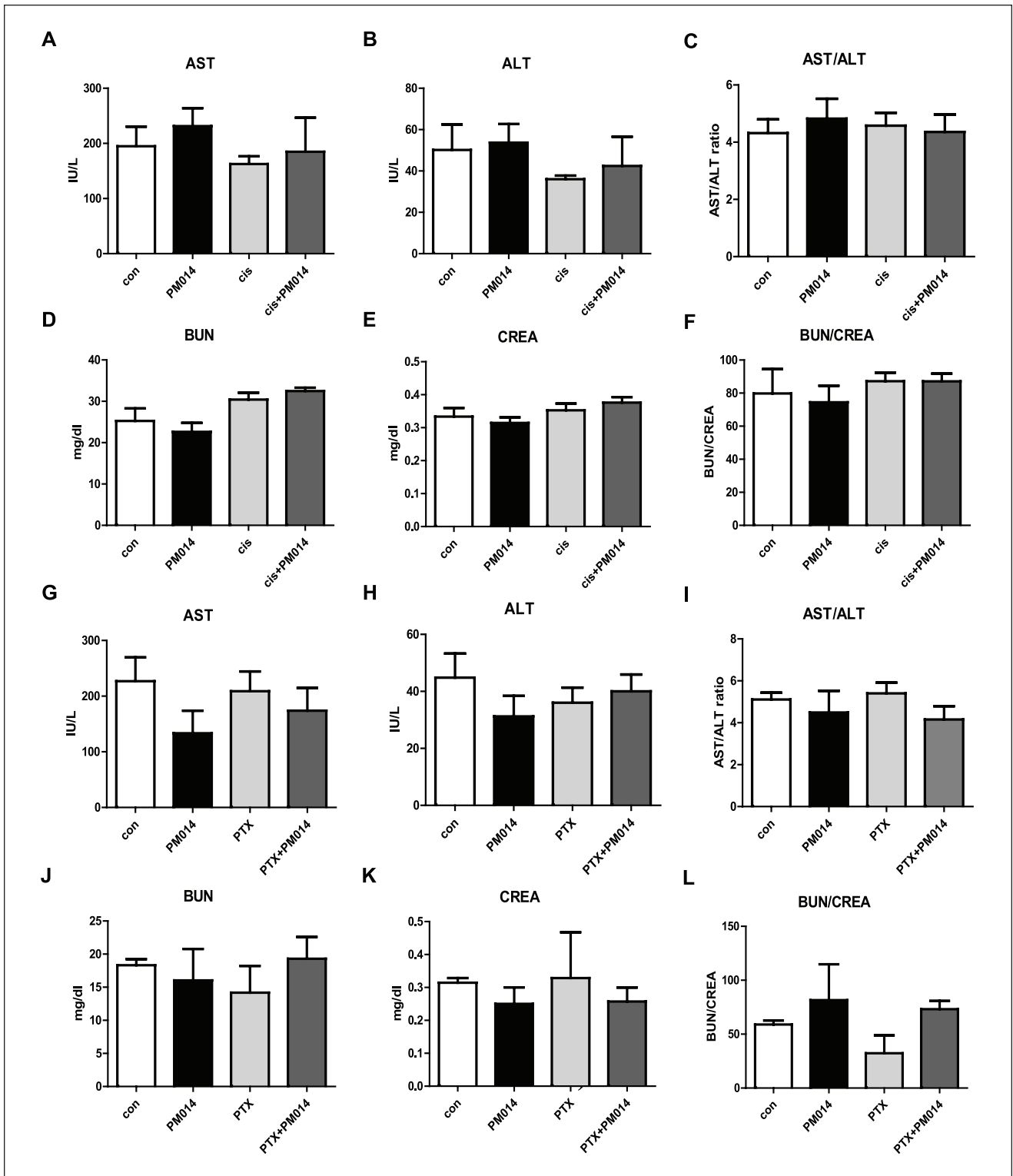


Figure 3. The effect of PM014 on liver and kidney function in cisplatin or paclitaxel-treated tumor-bearing mice. Biochemical analysis was performed in blood serum from mice treated phosphate-buffered saline (PBS) or PM014 with or without (A-F) cisplatin or (G-L) paclitaxel. The level of serum (A, G) alanine aminotransferase (AST), (B, H) aspartate aminotransferase (ALT), and (C, I) AST/ALT ratio were measured to assess liver damage and the level of serum (D, J) blood urea nitrogen (BUN), (E, K) creatinine (CREA), and (F, L) BUN/CREA were examined to assess kidney function. All data are presented as the means \pm SEMs.

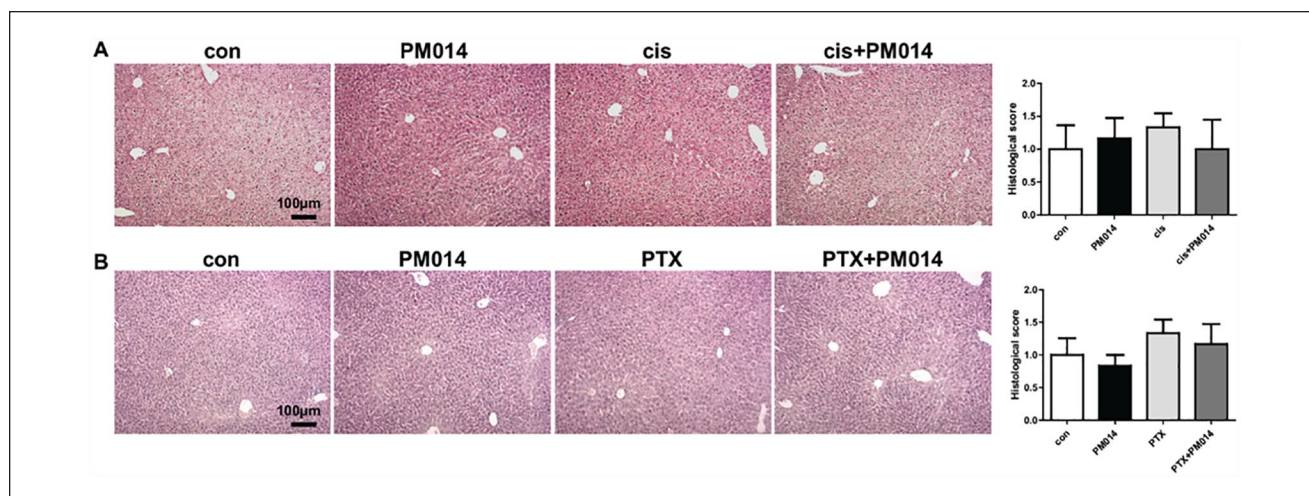


Figure 4. Liver histology after treatment with cisplatin or paclitaxel and PM014. Representative hematoxylin and eosin (H&E)-stained sections (left) and the histological scores (right) of liver tissue from mice treated phosphate-buffered saline (PBS) or PM014 with or without (A) cisplatin or (B) paclitaxel. Total magnification, $\times 10$ (scale bar: 100 μm). The scores are expressed as the means \pm SEMs.

difference between the control and cis group. In addition, cis + PM014 did not alter the renal state compared with cis alone (32.48 ± 0.81 mg/dL in cis + PM014). The level of CREA did not differ among the 4 groups (0.33 ± 0.03 mg/dL in control; 0.31 ± 0.02 mg/dL in PM014; 0.35 ± 0.02 mg/dL in cis; 0.38 ± 0.02 mg/dL in cis + PM014; Figure 3D and E). Similarly, the BUN/CREA ratio did not differ among the groups (Figure 3F).

In the PTX model, the mean level of AST and ALT did not significantly change, but AST was slightly decreased in the PM014 and PTX + PM014 treatments compared with the control group (AST = 226.83 ± 42.69 IU/L and ALT = 44.83 ± 8.46 IU/L in control; AST = 133.40 ± 40.43 IU/L and ALT = 31.20 ± 7.21 IU/L in PM014; AST = 208.83 ± 35.64 IU/L and ALT = 36.00 ± 5.34 IU/L in PTX; AST = 173.83 ± 40.75 IU/L and ALT = 40.00 ± 5.92 IU/L in PTX + PM014; Figure 3G and H). No differences were detected in the AST/ALT ratio (Figure 3I). The levels of renal enzymes BUN and CREA did not differ among the 4 groups (BUN = 18.33 ± 2.41 mg/dL and CREA = 0.31 ± 0.01 mg/dL in control; BUN = 16.03 ± 4.77 mg/dL and CREA = 0.25 ± 0.05 mg/dL in PM014; BUN = 14.17 ± 4.06 mg/dL and CREA = 0.33 ± 0.14 mg/dL in PTX; BUN = 19.30 ± 3.29 mg/dL and CREA = 0.26 ± 0.04 mg/dL in PTX + PM014; Figure 3J and K), and the BUN/CREA ratio showed no changes (Figure 3L). Together, these data showed that 200 mg/kg of PM014 did not cause hepatocellular or renal injury in the cis/PTX-treated tumor-bearing mouse model.

Histological Analysis

To ensure that PM014 did not affect hepatocellular function, histological changes were examined by hematoxylin

and eosin staining. The control and PM014-treated mice did not show necrosis, inflammation, or degeneration, which is observed as foamy cells or swellings. Hepatic injury was not observed either the cis or cis + PM014 groups. The histological scores did not significantly differ among the groups (Figure 4A). PTX and PTX + PM014 also showed a normal liver cell appearance and the histological scores showed no significance among the groups (Figure 4B).

Kidney histopathological examination revealed that the control and PM014 groups had normal glomeruli and renal tubule structure. Although the BUN and CREA levels were in the normal range, the cis-treated group presented weak atrophy in the glomeruli with slightly widened capsular spaces. Weak damage was observed in the tubules with an unclear brush border. In addition, immune cell infiltration was increased by cis injection compared with control; however, the coadministration of cis and PM014 did not aggravate the renal damage (Figure 5A). In the PTX model, although partial immune cell infiltration was observed, there were neither widened capsular spaces nor atrophy of the tubular structure. The histopathological score confirmed the absence of severe renal damage in all groups (Figure 5B). Thus, the coadministration of either chemotherapeutic agent or PM014 did not aggravate renal atrophy.

Inhibitory Effects of PM014 Toward the 9 CYP Isoforms in Human Liver Microsomes

The inhibitory effects by PM014 extract on the activity of the 9 major CYPs isoforms (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) in human liver microsomes are illustrated in Figure 6. The IC_{50} (inhibitory concentration) values for the known potent

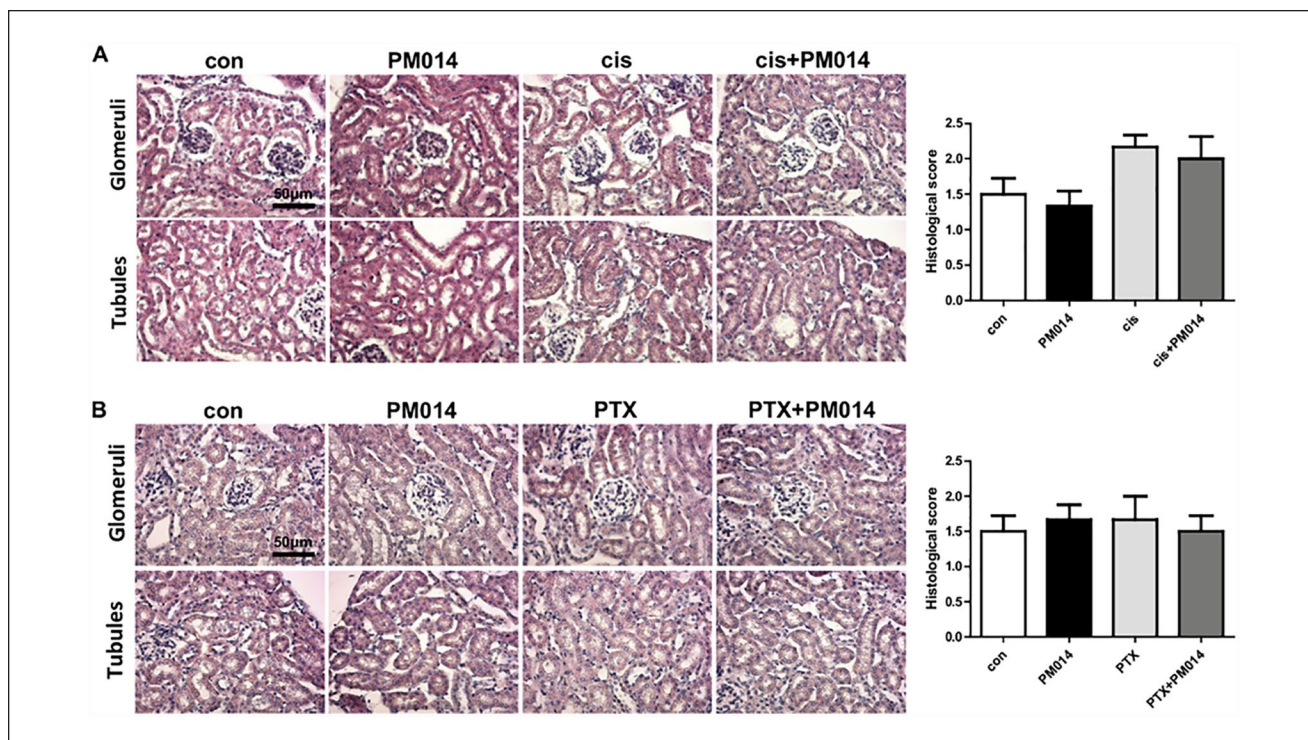


Figure 5. Histopathological analysis of kidney after treatment with cisplatin or paclitaxel and PM014. Representative hematoxylin and eosin (H&E)-stained renal sections (left); glomeruli, upper panel; tubules, bottom panel; and histological scores (right) of kidney tissue from mice treated phosphate-buffered saline (PBS) or PM014 with or without (A) cisplatin or (B) paclitaxel. Total magnification, $\times 40$ (scale bar: 50 μm). The scores are expressed as the means \pm SEMs.

inhibitors used in the inhibition studies were similar to previously reported values.²² The PM014 extract had no apparent inhibitory effects on any of the 9 tested CYPs; the residual enzyme activities at the highest tested concentration (200 $\mu\text{g}/\text{mL}$) were greater than 80%, except for CYP2A6 (58.7%) and UGT1A3 (30.8%) in the presence of PM014 extract (Figure 6). These results implied that the PM014 extract is unlikely to cause a significant metabolic drug-drug interaction via inhibition of any of the 9 major human hepatic CYPs involved in drug metabolism.

Discussion

In this study, we demonstrated the efficacy of coadministration of herbal formula PM014 and chemotherapies *in vivo*. Our previous study revealed that PM014 successfully inhibited lung fibrosis by reducing inflammatory cell infiltration in the lung airway in damaged pulmonary mouse model.^{19,20} The most efficient concentration of PM014 was 200 mg/kg, which had a therapeutic effect to inhibit the inflammation. No inhibitory effect was observed when PM014 was administered once a week as a single injection or for 2 weeks in a total of 2 injections, while chronic treatment showed significant effects. In the present study, we followed the same experimental schedule of chronic

treatment by administering PM014 every 2 days. As a result, 200 mg/kg of PM014 did not interrupt the antitumor effect of cis and PTX and showed no negative effect on hepatic or renal function in tumor-bearing mice model, suggesting that PM014 and anticancer drugs may be used as a promising combination therapy.

Toxicity is an important issue in combination therapy. PM014 treatment (200 mg/kg) did not show hepatotoxicity or nephrotoxicity when used alone in both the cis and PTX models. Although cis + PM014-treated mice showed weak damage in the renal cells, this damage was caused by cis as the coadministration of PM014 after cis treatment did not worsen the inflammation or cell damage. No functional damage to the liver or kidney was observed in the PTX + PM014 treated mice; however, as PM014 did not show a protective effect on nephrotoxicity, the use of a safe dose of cis could be critical in this combination therapy. For an accurate safety measurement, we previously examined the toxicity profile of PM014 in a rat model.²³ PM014 was orally treated daily at dosage levels of 750, 1500, and 3000 mg/kg repeatedly for 13 weeks. After long-term treatment of PM014, no toxicity was observed in both male and female rats. PM014 did not change body weight, food consumption, hematological parameters (18 parameters), and serum parameters (20 parameters) including the parameters on

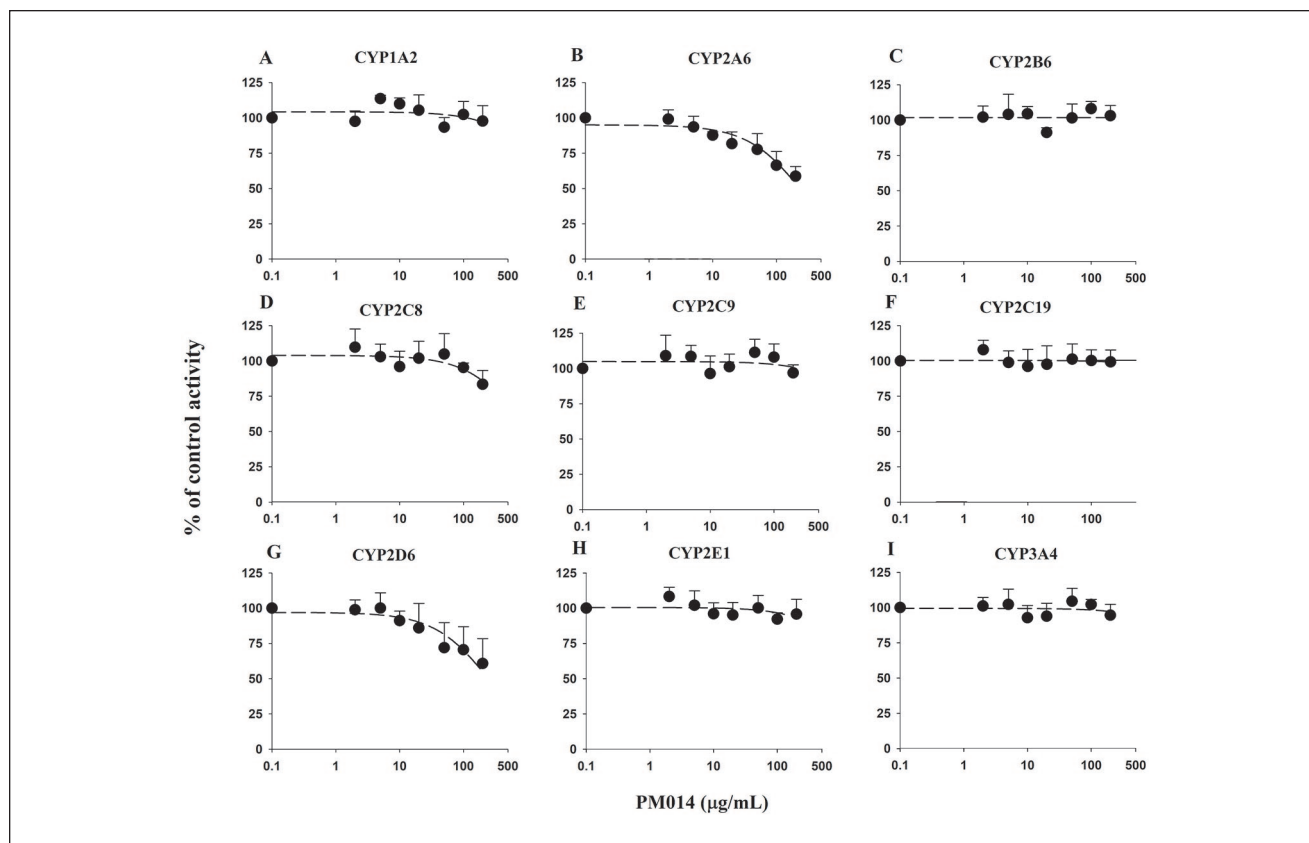


Figure 6. IC_{50} curves of PM014 for the 9 human P450 activities using the cocktail assays. CYP1A2 for phenacetin *O*-deethylase (A), CYP2A6 for coumarin 7-hydroxylase (B), CYP2B6 for bupropion hydroxylase (C), CYP2C8 for rosiglitazone *p*-hydroxylase (D), CYP2C9 for tolbutamide 4-hydroxylase (E), CYP2C19 for *S*-mephenytoin 4-hydroxylase (F), CYP2D6 for dextromethorphan *O*-demethylase (G), CYP2E1 for chlorzoxazone 6-hydroxylase (H), and CYP3A4 for midazolam 1'-hydroxylase (I). Data are the mean \pm SD of triplicate determinations. The dashed lines represent the best fit to the data with nonlinear regression.

inflammatory responses. Furthermore, PM014 did not have any direct effect on organ weight per body weight index (18 parameters including adrenal gland, ovary, thymus, spleen, kidney, heart, lung, brain, and liver) and neither induced morphological changes, indicating that there was no systemic or toxicological changes induced by PM014.

Potential drug-drug interactions in multiple drug therapies that may block the original effect and may cause unexpected side effects are also critical in combination therapies. We showed that PM014 did not affect the anticancer activity of cis or PTX, suggesting that PM014 can be used in potential cancer combination therapies to inhibit the side effects of chemotherapy or radiotherapy. As regarding a pharmacokinetic drug-drug interaction, Agergaard et al²⁴ reported that coadministration of the widely used anticoagulant drug clopidogrel can lead to the increased neurotoxicity of PTX, and this might be attributed to the inhibition of CYP2C8-mediated PTX metabolism. In this study, we demonstrated that PM014 showed negligible inhibitions toward the 9 major CYP enzymes including the CYP2C8 in human liver microsomes. Based on these observations, it is unlikely

that PM014 alter the pharmacokinetics of drugs metabolized by the tested 9 CYPs. These findings provide some useful information for the safe use of PM014 as a combination agent in clinical practice.

Chemotherapy has been used to enhance the effect of radiation damage in tumors.²⁵⁻²⁷ The platinum anticancer drugs such as cis, carboplatin, and oxaliplatin have been commonly used as radio-sensitizers.²⁸⁻³⁰ PTX also has been reported to have sensitization properties.³¹⁻³³ In clinical use, the chemotherapeutic agents cis and PTX have been used in the range of 20 to 60 mg/m² (low dose) before or after irradiation to cure lung cancer either weekly at or 2-week intervals long term.^{34,35} The total dose of chemotherapeutic agent in this study, 10 mg/kg, was calculated using conversion factor and is equal to approximately 30 mg/m² of human therapeutic dose range, which is an effective dose range in patients, but long-term mouse study is further needed to clarify the safe use relevant to humans. Moreover, it has been reported that a sustained interval of chemotherapy resulted in enhanced therapeutic effect.³⁶ Wang et al³⁷ reported that a low dose of PTX inhibits the EMT

and ameliorates pulmonary fibrosis. Although 10 mg/kg chemotherapeutic agent successfully reduced the LLC tumor growth in this study, a long-term study with sustained injection in a low dose is needed to understand the efficacy of anticancer drug and PM014 coadministration in a pulmonary fibrosis with lung cancer model.

In conclusion, PM014 did not block the antitumor activity of cis and PTX and coadministration of PM014 and antitumor agents did not induce hepatic or renal injury. Taken together, the results presented here suggest that PM014 is compatible with cis or PTX treatment in lung cancer therapy without promoting side effects. Thus, PM014 may be effectively used as combination agent in cancer treatment.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was supported by grants from the National Research Foundation of Korea (NRF) funded by the Korea government (NRF-2020R1A2B5B03002164).

ORCID iD

Hyunsu Bae  <https://orcid.org/0000-0002-0299-3582>

References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin.* 2017;67:7-30.
2. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer.* 2013;49:1374-1403.
3. Curran WJ Jr, Paulus R, Langer CJ, et al. Sequential vs. concurrent chemoradiation for stage III non-small cell lung cancer: randomized phase III trial RTOG 9410. *J Natl Cancer Inst.* 2011;103:1452-1460.
4. Leo F, Pelosi G, Sonzogni A, Chilosi M, Bonomo G, Spaggiari L. Structural lung damage after chemotherapy fact or fiction? *Lung Cancer.* 2010;67:306-310.
5. Torrasi JM, Schwartz LH, Gollub MJ, Ginsberg MS, Bosl GJ, Hricak H. CT findings of chemotherapy-induced toxicity: what radiologists need to know about the clinical and radiologic manifestations of chemotherapy toxicity. *Radiology.* 2011;258:41-56.
6. Limper AH. Chemotherapy-induced lung disease. *Clin Chest Med.* 2004;25:53-64.
7. Vander Els NJ, Stover DE. Chemotherapy-induced lung disease. *Clin Pulm Med.* 2004;11:84-91.
8. Vahid B, Marik PE. Pulmonary complications of novel antineoplastic agents for solid tumors. *Chest.* 2008;133:528-538.
9. Wittram C, Mark EJ, McLoud TC. CT-histologic correlation of the ATS/ERS 2002 classification of idiopathic interstitial pneumonias. *Radiographics.* 2003;23:1057-1071.
10. Leslie KO, Gruden JF, Parish JM, Scholand MB. Transbronchial biopsy interpretation in the patient with diffuse parenchymal lung disease. *Arch Pathol Lab Med.* 2007;131:407-423.
11. Roach M 3rd, Gandara DR, Yuo HS, et al. Radiation pneumonitis following combined modality therapy for lung cancer: analysis of prognostic factors. *J Clin Oncol.* 1995;13:2606-2612.
12. Barriger RB, Fakiris AJ, Hanna N, Yu M, Mantravadi P, McGarry RC. Dose-volume analysis of radiation pneumonitis in non-small-cell lung cancer patients treated with concurrent cisplatin and etoposide with or without consolidation docetaxel. *Int J Radiat Oncol Biol Phys.* 2010;78:1381-1386.
13. Graves PR, Siddiqui F, Anscher MS, Movsas B. Radiation pulmonary toxicity: from mechanisms to management. *Semin Radiat Oncol.* 2010;20:201-207.
14. Taghian AG, Assaad SI, Niemierko A, et al. Risk of pneumonitis in breast cancer patients treated with radiation therapy and combination chemotherapy with paclitaxel. *J Natl Cancer Inst.* 2001;93:1806-1811.
15. Roh GS, Seo SW, Yeo S, et al. Efficacy of a traditional Korean medicine, Chung-Sang-Bo-Ha-Tang, in a murine model of chronic asthma. *Int Immunopharmacol.* 2005;5:427-436.
16. Lee H, Kim Y, Kim HJ, et al. Herbal formula, PM014, attenuates lung inflammation in a murine model of chronic obstructive pulmonary disease. *Evid Based Complement Alternat Med.* 2012;2012:769830.
17. Jung KH, Haam KK, Park S, et al. The standardized herbal formula, PM014, ameliorated cigarette smoke-induced lung inflammation in a murine model of chronic obstructive pulmonary disease. *BMC Complement Altern Med.* 2013;13:219.
18. Jung KH, Choi HL, Park S, et al. The effects of the standardized herbal formula PM014 on pulmonary inflammation and airway responsiveness in a murine model of cockroach allergen-induced asthma. *J Ethnopharmacol.* 2014;155:113-122.
19. Kim JY, Shin D, Lee G, et al. Standardized herbal formula PM014 inhibits radiation-induced pulmonary inflammation in mice. *Sci Rep.* 2017;7:45001.
20. Kim KH, Lee S, Lee H, et al. A standardized herbal extract PM014 ameliorates pulmonary fibrosis by suppressing the TGF- β 1 pathway. *Sci Rep.* 2018;8:16860.
21. Lee T, Park JY, Lee HY, et al. Lung cancer in patients with idiopathic pulmonary fibrosis: clinical characteristics and impact on survival. *Respir Med.* 2014;108:1549-1555.
22. Zheng YF, Bae SH, Choi EJ, et al. Evaluation of the in vitro/in vivo drug interaction potential of BST204, a purified dry extract of ginseng, and its four bioactive ginsenosides through cytochrome P450 inhibition/induction and UDP-glucuronosyltransferase inhibition. *Food Chem Toxicol.* 2014;68:117-127.
23. Chung HS, Lee H, Bae H. Thirteen-week study of PM014 subchronic oral toxicity in rats. *Evid Based Complement Alternat Med.* 2014;2014:189673.

24. Agergaard K, Mau-Sorensen M, Stage TB, et al. Clopidogrel-paclitaxel drug-drug interaction: a pharmacoepidemiologic study. *Clin Pharmacol Ther.* 2017;102:547-553.
25. Jett JR. Current treatment of unresectable lung cancer. *Mayo Clin Proc.* 1993;68:603-611.
26. Bayman NA, Blackhall F, Jain P, Lee L, Thatcher N, Faivre-Finn C. Management of unresectable stage III non-small-cell lung cancer with combined-modality therapy: a review of the current literature and recommendations for treatment. *Clin Lung Cancer.* 2008;9:92-101.
27. von Dincklage JJ, Ball D, Silvestri GA. A review of clinical practice guidelines for lung cancer. *J Thorac Dis.* 2013;5(suppl 5):S607-S622.
28. Golding RP, van Zanten TE. Radiation and platinum drug interaction. *Int J Radiat Biol Relat Stud Phys Chem Med.* 1986;50:945-946.
29. Schaake-Koning C, van den Bogaert W, Dalesio O, et al. Effects of concomitant cisplatin and radiotherapy on inoperable non-small-cell lung cancer. *N Engl J Med.* 1992;326:524-530.
30. Begg AC, van der Kolk PJ, Emond J, Bartelink H. Radiosensitization in vitro by cis-diammine (1,1-cyclobutanedicarboxylato) platinum(II) (carboplatin, JM8) and ethylenediammine-malonatoplatinum(II) (JM40). *Radiother Oncol.* 1987;9:157-165.
31. Akerley W, Choy H. Single-agent paclitaxel and radiation for non-small cell lung cancer. *Semin Radiat Oncol.* 1999;9(2 suppl 1):85-89.
32. Liebmann J, Cook JA, Fisher J, Teague D, Mitchell JB. In vitro studies of Taxol as a radiation sensitizer in human tumor cells. *J Natl Cancer Inst.* 1994;86:441-446.
33. Hiro J, Inoue Y, Toiyama Y, et al. Possibility of paclitaxel as an alternative radiosensitizer to 5-fluorouracil for colon cancer. *Oncol Rep.* 2010;24:1029-1034.
34. Havemann K, Wolf M, Görg C, Hackenbruch E, Pfab R, Görg K. Preclinical and clinical experience with cisplatin and carboplatin and simultaneous radiation in non-small cell lung cancer. *Ann Oncol.* 1992;3(suppl 3):S33-S37.
35. Ettinger DS, Aisner DL, Wood DE, et al. NCCN guidelines insights: non-small cell lung cancer, version 5.2018. *J Natl Compr Canc Netw.* 2018;16:807-821.
36. Edinger M, Cao YA, Verneris MR, Bachmann MH, Contag CH, Negrin RS. Revealing lymphoma growth and the efficacy of immune cell therapies using in vivo bioluminescence imaging. *Blood.* 2003;101:640-648.
37. Wang C, Song X, Li Y, et al. Low-dose paclitaxel ameliorates pulmonary fibrosis by suppressing TGF- β 1/Smad3 pathway via miR-140 upregulation. *PLoS One.* 2013;8:e70725.