*J. Microbiol. Biotechnol.* (2008), **18**(2), 369–376

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY



# Radioprotective Effect of Cyclo(L-Phenylalanyl-L-Prolyl) on Irradiated Rat Lung

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Received: June 28, 2007 / Accepted: September 6, 2007

**In the present study, we investigated the radioprotective effect of cyclo(L-phenylalanyl-L-prolyl) on irradiated rat lungs to determine its potential as a radioprotective agent. We found that early lung damage induced by irradiation was reduced by treatment with 40 mg/kg of cyclo(L-phenylalanyl-L-prolyl) in the latent and early pneumonitis phases. Expression of TNF-α and TGF-β1 at 2 and TGF-β1 at 8 weeks post-irradiation was decreased in animals that received both radiation and cyclo(L-phenylalanyl-L-prolyl) compared with animals that received radiation alone. Evidence indicated that the proinflammatory cytokine TNF-α and the fibrogenic cytokine TGF-β1 likely play a role in the radioprotective effect of cyclo(L-phenylalanyl-L-prolyl). However, besides TNF-α and TGF-β1 expressions, the precise mechanism by which cyclo(L-phenylalanyl-L-prolyl) ameliorates the induced radiation damage is not clear.**

**Keywords:** Cyclo(L-phenylalanyl-L-prolyl), radioprotective agent, TNF-α, TGF-β1

Radiation therapy has been used in the treatment of cancer patients for almost a hundred years. It is most commonly used for treating primary and metastatic tumors. Typically, the radiation beam is focused on a finite number of known or suspected tumors, and the radiation dose and path are designed to minimize damage to the surrounding, non-target, and normal tissue. Nevertheless, radiation therapy for the treatment of primary or metastatic tumors of the lung or other carcinomas of the chest is hampered by diffuse infiltration of the lung by secondary metastases of the primary lung cancer. To minimize damage to the organs of the chest, including the lung, during radiation treatment, it is necessary to use radioprotective agents. These agents can be low-molecular-weight compounds, such as free-radical scavengers or the antioxidants, vitamin E and ascorbic acid [25, 26, 43]. Sulfhydryl compounds and cationic thiols have also been evaluated for use as chemoactive radioprotective agents [8]. Examples include S-2-(3-aminopropylamino) ethyl phosphorothioic acid (WR2721), a thiophosphate derivative of aminothiol cysteamine, and 2-(3-aminopropyl) aminoethanethiol (WR1065) [10, 19, 20, 22, 36, 40, 44]. Some radioprotective agents are high-molecular-weight molecules. These include biological molecules that have antioxidant properties, such as superoxide dismutase, which destroys oxygen anions generated during radiation and reduces the concentration of mediators of radiation damage, as well as glucocorticoids, vasodilators, and angiotensin-1-converting enzyme (ACE) inhibitors [5–7, 17, 43]. Among the ACE inhibitors, Captopril has been widely studied and has been shown to be effective in protecting the kidneys, lungs, and heart from radiation-induced damage [39, 48]. The mechanism of this protective effect, however, is unclear.

The clinical response to radiation-induced lung damage includes a latent period, followed by an acute inflammatory phase, an intermediate phase, and fibrosis, with different pathological changes associated with each stage. The lung cells targeted by radiation are alveolar epithelial cells and vascular endothelial cells [34].

In the current study, pulmonary fibrosis was found to be associated with early and persistent elevation of cytokine production following pulmonary irradiation. The temporal relationship between the elevation of specific cytokines and the histological and biochemical evidence of fibrosis serves to illustrate the continuum of responses that underlie radiation-induced pulmonary damage, and supports the concept of a perpetual cascade of cytokines produced in response to irradiation [35].

TNF-α, interleukin (IL)-1α, and IL-1β mRNA levels in mice are increased after irradiation during the latent period, and then decreased during the late pneumonic phase. TGF-α1 expression is increased 2 weeks post-irradiation, persists until 8 weeks, and then returns to baseline levels [11, 15, 23, 46]. These results suggest that changes in the levels of

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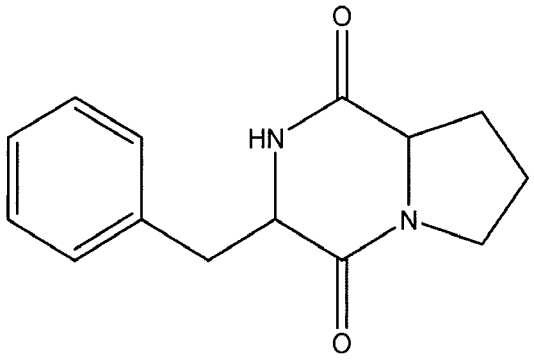


**Fig. 1.** Chemical structure of cyclo(L-phenylalanyl-L-prolyl).

these cytokines, particularly TNF-α and TGF-β1, represent a useful index for screening and developing radioprotective agents for radiotherapy.

During the course of an antibiotics screening program in our laboratory, we isolated an anti-VRE compound, cyclo(L-phenylalanyl-L-prolyl), from cultures of *Streptomyces* sp. AMLK-335 (Fig. 1), and found that it has both anti-VRE activity and inhibits topoisomerase I [30, 33]. In the current study, we investigated histological changes and the expression of TNF-α and TGF-β1 over 10 weeks in rat lungs following irradiation, and identified a novel radioprotective activity of cyclo(L-phenylalanyl-L-prolyl).

## Materials and Methods

### Animals

Adult male normotensive Sprague-Dawley rats (Daehan Laboratory Animals, Korea), weighing 200–250 g, were used in all experiments. All animals were maintained at a temperature of 20°C to 23°C, and given food (Purina rodent chow, Korea) and water *ad libitum*.

### *In Vitro* Radioprotection Assay

For radioprotection, SRB assay was used [44]. NCI-H1437 cells were maintained in RPMI 1640 (Irvine Scientific, Santa Ana, CA, U.S.A.) with 10% fetal bovine serum (Gemini, Woodland, CA, U.S.A.) and penicillin (100 units/ml)/streptomycin (100 μg/ml) (complete growth medium) [3]. Cells were harvested by trypsinization, counted, and plated in 4-wells plates. After pretreatment with cyclo(L-phenylalanyl-L-prolyl) and amifostine (WR2721) as positive control agent for 48 hours, plates were irradiated by 6 Gy (Cs137), and media were immediately removed, washed 3 times with phosphate-buffered saline (PBS), and replaced with fresh medium. Culture medium was replaced with fresh medium every 3 days. Recovery time was 8 days. Results were expressed as relative percentages of absorbance as compared with untreated controls.

### DPPH Assay

Free-radical scavenging activity of cyclo(L-phenylalanyl-L-prolyl) was measured using the method of Brand-Williams *et al.* [2] with some modification. A 0.1 mM solution of DPPH (1,1-diphenyl-2-picryl-hydrazyl) in methanol was prepared and 4 ml of this solution was added to 0.2 ml of cyclo(L-phenylalanyl-L-prolyl). The decrease in absorbance at 517 nm was measured after 60 min. For control, 0.2 ml of distilled water was added instead of cyclo(L-phenylalanyl-L-prolyl). Free-radical scavenging activity of cyclo(L-phenylalanyl-L-prolyl) was compared with that of butylated hydroxyanisole (BHA) and amifostine as positive control. Free-radical scavenging activity was expressed as the percentage of DPPH decrease.

### Radiation and Cyclo(L-Phenylalanyl-L-Prolyl) Treatment

Rats were divided into three groups: no-radiation, radiation-alone, and a combination of radiation and cyclo(L-phenylalanyl-L-prolyl). After being anesthetized with sodium pentobarbital (30 mg/kg, i.p.), rats received a single 12.5 Gy (2 Gy/min) of radiation in the left lung using a 6 MV linear accelerator (NEC 1006X, Japan).

Rats in the radiation-alone group consumed water, and the combination group consumed water containing cyclo(L-phenylalanyl-L-prolyl) after irradiation. Cyclo(L-phenylalanyl-L-prolyl) mixed with water was given orally on a continuous basis from 3 days prior to irradiation up to the 10th week after exposure. Water consumption was measured weekly, and was found to be constant at approximately 40 mg/kg/day in the treatment group.

### Histopathology

Rats were sacrificed 2 and 10 weeks after irradiation. At the time of death, the lungs from each rat were bisected and immediately placed in 10% buffered formalin. Specimens from each animal were embedded in paraffin and sectioned into 4-micron (m)-thick sections. Sections were stained by hematoxylin eosin or Masson Trichrome, and then examined by light microscopy.

### Measurement of TNF-α and TGF-β1

The peroxidase-antiperoxidase method was used for immunohistochemical analysis, as previously described [12]. Briefly, tissues were immediately preserved in tissue freezing medium, and then slides were fixed in a 70% acetone-30% methanol solution for 10 min, air-dried, and then washed three times in PBS for 10 min. Specimens were incubated in 10% goat serum for 1 h at room temperature, and then endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide for 10 min at room temperature. Before incubation with anti-TNF-α (PromoCell, Heidelberg, Germany) or anti-TGF-β1 (Santa Cruz Biotechnology, U.S.A.) antibody, slides were washed with distilled water and soaked in PBS for 5 min. Immunoreactive proteins were detected using the LSAB peroxidase-antiperoxidase reaction kit (Dako), and visualized using 3-aminoethyl 9-carbasol (ACE) color reagent and Meyer's hematoxylin.

### Histopathological Evaluation

A subjective evaluation of each slide was used to assign a rating of 1+ to 3+ to each component of the organ under examination; 1+= minimal damage, 2+=severe damage, and 3+=extensive damage (Table 1).

## Results

### Radioprotection on NCI-H1437 Cells

*In vitro* radioprotective effect was shown by survival rate. The radiation induced varying extents of damage on NCI-

**Table 1.** Grading of histopathological findings in irradiated rat lung.

| |
|---|
| Parenchymal collapse, consolidation, hemorrhage, and alveolar epithelial cell changes |
| 1+: less than 10% of lung parenchymal changes |
| 2+: 10–50% of lung parenchymal changes |
| 3+: more than 50% of lung parenchymal changes |
| Bronchial epithelium |
| 1+: mild proliferation of epithelium |
| 2+: moderate proliferation with papillary configuration |
| 3+: marked proliferation of epithelium with detachment of epithelial cells |
| Perivascular edema |
| 1+: mild accumulation of edema fluid |
| 2+: moderate accumulation of fluid |
| 3+: marked accumulation of fluid with fibrin deposition |
| Blood vessels |
| 1+: vacuolization of vascular endothelial cells |
| 2+: vacuolization of vascular muscle layer |
| 3+: marked degeneration of vascular wall or thickening of muscle layer with narrowing of the vascular lumen |

H1437 cells. The range of survival rates showed 36% on NCI-H1437 cells by 6 Gy radiation. However, cyclo(L-phenylalanyl-L-prolyl) and amifostine (WR2721) affected the recovery of radiation-induced damage in a dose-dependent manner. The survival rate after pre-treatment with cyclo(L-phenylalanyl-L-proiyl) and amifostine (WR2721) was increased by more than twice that with radiation alone. Amifostine, which is a well-known radioprotector, showed 57% of survival rate at the dose of 100 μM, although cyclo(L-phenylalanyl-L-proiyl) at the same dose showed more effective protection on NCI-H1434 cells than amifostine.

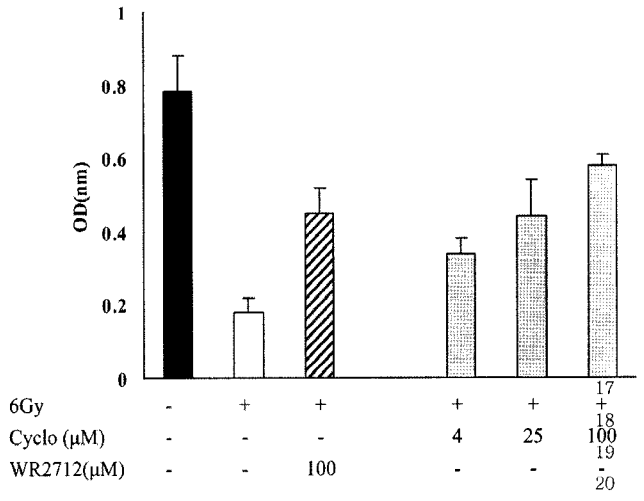


**Fig. 2.** Radioprotection (60Co, 4 Gy) in NCI-H1437 cell line treated with WR2721 (100 μM) and cyclo(L-phenylalanyl-L-prolyl) (4, 25, and 100 μM), respectively.

**Table 2.** Free-radical scavenging activity of cyclo(L-phenylalanyl-L-prolyl) and amifostine by DPPH assay.

| | Cyclo(pro-phe) | Amifostine | BHA |
|---|---|---|---|
| $IC_{50}$ (μM) | 24 | 27 | 50 |

Cyclo(pro-phe), cyclo(L-phenylalanyl-L-prolyl); BHA, butylated hydroxyanisole.

Cyclo(L-phenylalanyl-L-prolyl) at 4, 20, and 100 μM showed the survival rates of 43%, 56%, and 74%, repectively, against radiation-induced damaged cell line by 6 Gy radiation. When the cells were treated with cyclo(L-phenylalanyl-L-prolyl) alone, no effect on the growth was observed, compared with untreated cells (Fig. 2).

### Free-Radical Scavenging Effect of Cyclo(L-Phenylalanyl-L-Prolyl)

Table 2 shows comparative free-radical scavenging activities of cyclo(L-phenylalanyl-L-prolyl) with other well-known antioxidant agents such as BHA and amifostine, using DPPH assay. As seen in the table, 24 μM of cyclo(L-phenylalanyl-L-prolyl) was as effective as 27 μM amifostine, evidenced by DPPH assay.

### Control, No-Radiation (Normal) Group

Experimental specimens from the lungs of animals in the control group, which received no treatment, were unremarkable in their histological features, and there were no differences or morphological changes between the right and left lungs of sham-irradiated animals.

### Histological Assessment of Radiation-induced Damage in the Rat Lung

Based on histopathology, animals in the radiation-alone group displayed more severe lung damage, including hemorrhaging into the alveolar space, changes in the alveolar and bronchial epithelium and blood vessels, and perivascular edema, compared with the combination radiation plus cyclo(L-phenylalanyl-L-prolyl) group at 2 weeks post-irradiation. Specifically, we found severe parenchymal patch consolidation and alveolar epithelial edema, due to focal hemorrhaging in the lungs of the radiation-alone group. In contrast, in animals that also received cyclo(L-phenylalanyl-L-prolyl), thickening of the alveolar septum, epithelial cell injury, and changes of blood vessels were ameliorated compared with the radiation-alone group (Fig. 3, Table 3). Ten weeks post-irradiation, the blood vessels of animals in the radiation-alone group exhibited vacuolization, irregular thickening of the muscle layer, and detachment of endothelial cells, with extensive perivascular edema and fibrin deposition. The combination radiation plus cyclo(L-phenylalanyl-L-prolyl) group displayed less severe damage at 10 weeks compared with 2 weeks, displaying markedly decreased damage due to hemorrhaging into the alveolar

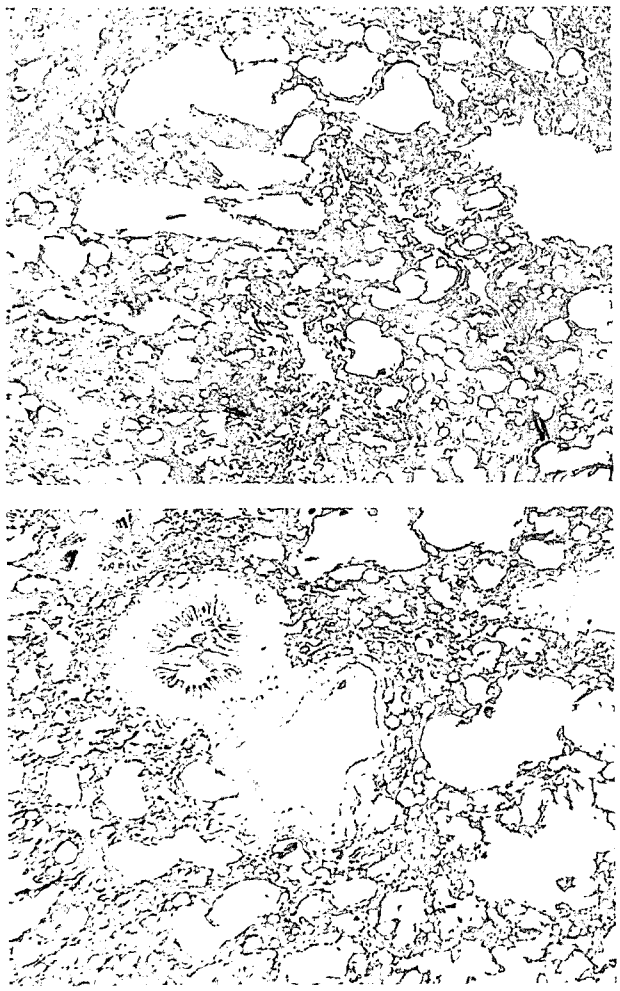

**Fig. 3.** Light micrograph of lung tissue at 2 weeks after 20 Gy irradiation (H&E stain, **A** and **B**; ×50). (**A**) 20 Gy radiation alone; (**B**) 20 Gy radiation plus cyclo(L-phenylalanyl-L-prolyl) 40 mg/kg.

space, changes in the alveolar and bronchial epithelium and blood vessels, and perivascular edema compared with the radiation-alone group (Fig. 4, Table 3).

### Immunohistochemical Analysis of Cytokine Expression in the Irradiated Rat Lung

The expression of TNF-α and TGF-β1 in the radiation-alone group was markedly increased in alveolar epithelial cells and macrophages of the alveolar septum compared with the normal group (data not shown). There were no differences in the levels of expression of TNF-α and TGF-β1 in lymphoid tissues 2 weeks post-irradiation, compared with the normal group. These changes in cytokine expression in most experimental tissue specimens of the radiation-alone group persisted up to 10 weeks post-irradiation, with the exception of TGF-β1 expression in the alveolar septum. TGF-β1 expression in the alveolar septum was greater at 10 weeks than at 2 weeks in the radiation-alone group.

The combination treatment group that received both radiation and cyclo(L-phenylalanyl-L-prolyl) exhibited a reduced effect on cytokine expression in all experimental tissue specimens. Two weeks post-irradiation, TNF-α and TGF-β1 expression in the combination group was markedly decreased in the alveolar epithelium, lymphoid tissue, and alveolar macrophages compared with the radiation-alone group. Furthermore, TGF-β1 expression was significantly reduced in the alveolar epithelium and alveolar macrophages. At 8 weeks, there was no significant difference in the expression of TNF-α and TGF-β1 in most experimental specimens between the radiation-alone group and the combination treatment group, with the exception of TGF-β1 in alveolar macrophages (Table 4).

## Discussion

A variety of cyclic dipeptides are present in protein and polypeptide hydrolysates, as well as in cultures of yeast and fungi. Simple cyclic dipeptides in nature exist free, or as a part of larger, more complex molecules, and they are enzymatically synthesized by several species of protists and

**Table 3.** Histopathological findings of left rat lung of the radiation-only group and the combined cyclo(pro-phe) and radiation group at 2 and 10 weeks after irradiation.

| | RT only (no.) | | | | Cyclo(pro-phe) and RT (no.) | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 weeks | | 10 weeks | | 2 weeks | | 10 weeks | |
| | ≤2+ | 3+ | ≤2+ | 3+ | ≤2+ | 3+ | ≤2+ | 3+ |
| Alveolar epithelial cell change | 4 | 6 | 9 | 1 | 8 | 2 | 10 | 0 |
| Blood vessels change | 1 | 9 | 2 | 6 | 9 | 1 | 9 | 1 |
| Bronchial epithelium change | 4 | 6 | 3 | 7 | 9 | 1 | 8 | 2 |
| Collapse & consolidation | 6 | 4 | 3 | 7 | 5 | 5 | 6 | 4 |
| Edema | 6 | 4 | 7 | 3 | 8 | 2 | 9 | 1 |
| Hemorrhage | 6 | 4 | 6 | 4 | 10 | 1 | 10 | 0 |
| Macrophage accumulation | 6 | 4 | 5 | 5 | 8 | 2 | 9 | 1 |
| Perivascular edema | 2 | 8 | 4 | 4 | 8 | 2 | 9 | 1 |
| Perivascular fibrosis | | | 8 | 2 | | | 10 | 0 |

RT, radiation; Cyclo(pro-phe), cyclo(L-phenylalanyl-L-prolyl).

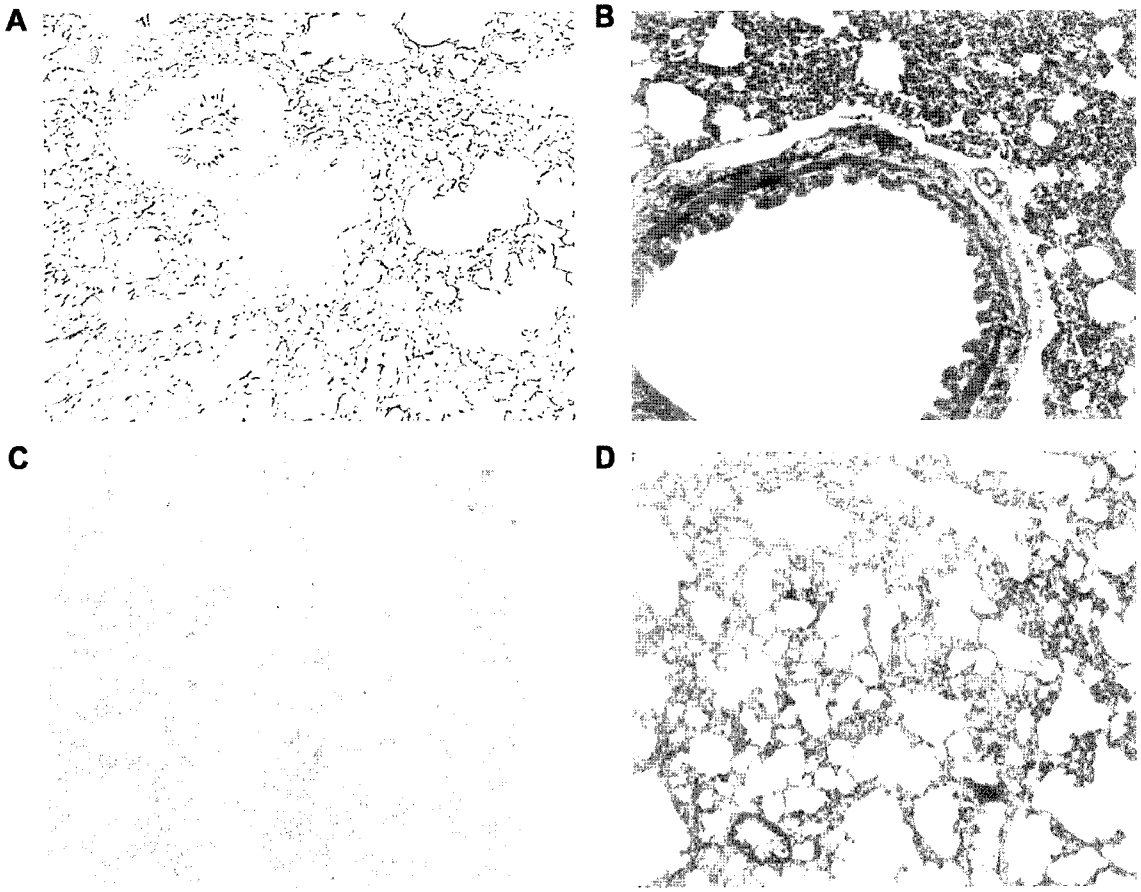


**Fig. 4.** Light micrograph of lung tissue at 10 weeks after 20 Gy irradiation (H&E stain, **A** and **B**; ×50. Masson-Trichrome stain, **C** and **D**; ×50). **A** and **C**, 20 Gy Radiation alone; **B** and **D**, 20 Gy radiation plus cyclo(L-phenylalanyl-L-prolyl) 40 mg/kg.

plants [24, 27]. Cyclo(L-leucyl-L-prolyl) is a cyclic dipeptide that is produced by *Rosellinia necatrix* and *Streptomyces gancidicus* [4, 13]. We isolated cyclo(L-leucyl-L-prolyl) from *Streptomyces* sp. KH-614, and found that it has anti-VRE (vancomycin-resistant Enterococci) activity towards a broad range of Enterococci. It also exhibits significant antitumor activity towards the leukemic cell lines K562, HL60, and U937 in an MTT assay [31, 32]. We also isolated another cyclic dipeptide, cyclo(L-phenylalanyl-L-prolyl), from *Streptomyces* sp. AMLK-335, and found that it has stronger inhibitory activity against topoisomerase I than campothecin, in a DNA relaxation assay using supercoiled plasmid (pBR322) DNA. Cyclo(L-phenylalanyl-L-prolyl) also exhibits antimicrobial activity against *E. faecalis* K-99-258 and K-98-637, which are vancomycin-resistant enterococci, *Staphylococcus aureus* TK784, *Saccharomyces cerervisiase* IFO1008, *Bacillus substilis* IAM1069, and *Micrococcus luteus* JCM1464 [32, 33].

**Table 4.** Changes of cytokine in the left rat lung of the radiation-only group and the combined cyclo(pro-phe) and radiation group at 2 and 10 weeks after irradiation.

| | | RT only (no.) | | | | | | Cyclo(pro-phe) and RT (no.) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 weeks | | | 10 weeks | | | 2 weeks | | | 10 weeks | | |
| | | ≤2+ | 3+ | * | ≤2+ | 3+ | * | ≤2+ | 3+ | * | ≤2+ | 3+ | * |
| TNFα | Lymphoid tissue | 4 | 6 | | 4 | 5 | 1 | 8 | 2 | | 6 | 4 | |
| | Macrophage | 2 | 8 | | 5 | 5 | | 7 | 3 | | 5 | 4 | |
| | Septal epithelium | 2 | 8 | | 5 | 4 | 1 | 9 | 1 | | 7 | 3 | |
| TGFβ1 | Lymphoid tissue | 4 | 5 | 1 | 7 | 1 | | 8 | 2 | | 5 | 5 | |
| | Macrophage | 2 | 7 | 1 | 1 | 6 | 1 | 9 | 1 | | 7 | 3 | |
| | Septal epithelium | 3 | 6 | 1 | 7 | 1 | | 9 | 1 | | 5 | 5 | |

RT, radiation; *, not reactive.
Cyclo(pro-phe), cyclo (L-phenylalanyl-L-prolyl).

To date, there are only a few biological studies of cyclodipeptides. Jia *et al.* [14] reported that a newly identified cyclodipeptide, cordycedipeptide A from the culture supernatants of *Cordyceps sinensis*, exhibits cytotoxicity against various tumor cell lines, such as L-929, A375, and Hela. Another group found that cyclo(L-leucyl-L-prolyl) from *Achromobacter xylosoxidans* inhibits aflatoxin production in *Aspergillus parasiticus*. They also found that *Achromobacter xylosoxidans* markedly inhibits production of norsolorinic acid, a precursor of aflatoxin, in *A. parasiticus*, and that some cyclodipeptides, such as cyclo(D-leucyl-D-prolyl) and cyclo(L-valyl-L-prolyl), show inhibitory activity towards fungi [47]. Repression by cyclo(L-leucyl-L-prolyl) involves transcription of the aflatoxin-related genes *aflR*, *hexB*, *pksL1*, and *dmtA*, and this is the first report of a cyclodipeptide that affects aflatoxin production [47]. These two studies together show that the biological activity of cyclopeptides depends on their structural components.

Compounds that have radioprotective activity are known as radioprotectants. They include chemicals such as adamantylamide dipeptide, carnosine, MIGI-K, thymogen, thymohexin, WR2721 and WR1065, *N*-glycylglycyl-*S*-acetylcysteamine trifluoroacetate, and aminoarylthiazoles [9, 38, 42]. The structures of chemicals that show potent radioprotective activity generally include a polypeptide, amine, cysteine, and a polyamine containing phenyl or napthyl groups. Moreover, similar to most radioprotectors, this radioprotective agent, amifostine (WR2721), is a prodrug, which is dephosphorylated to give rise to the active free thiol metabolite WR1065 by the membrane-bound enzyme alkalinephosphatase. The protection is achieved either through a reactive oxygen species scavenging activity, mainly by reaction of amifostine with hydroxyl radicals, by depletion of oxygen or by donation of hydrogen ions to free radicals [21]. We suggested that this compound could have radioprotective activity based on free-radical scavenging effect similar to amifostine. In the present study, we found that cyclo(L-phenylalanyl-L-prolyl) is potentially a free-radical scavenger *in vitro* as amifostine, and that cyclo(L-phenylalanyl-L-prolyl) has potent radioprotective activity on rat lungs treated with 12.5 Gy of radiation, even though we could not carry out comparative experiment with amifostine.

In the radiation damage, the pathology and cytokine expression profile in severe late radiation damage generally follow a distinct sequence of events: a latent period when no overt evidence of damage exists; an acute inflammatory phase; an intermediate recovery period; and a late phase when the onset of fibrosis becomes apparent. Lung injury in particular is often accompanied by an inflammatory response that results in the activation of resident macrophages and expansion of this population of cells through recruitment of new cells [1, 15, 18, 28, 29].

In the current study, we identified two factors that may play a role in mediating the interaction between alveolar macrophages and interstitial fibroblasts: TNF-α, a major factor in pulmonary fibrosis, that induces fibrogenic cytokines and attenuates many of the events observed during fibrosis, and was reduced more than other fibrogenic cytokines, and TGF-β, which is equally or more important than TNF-α in fibrosis [23, 24, 37, 41, 45]. Using immunohistochemical analysis, we demonstrated that cyclo(L-phenylalanyl-L-prolyl) reduced the level of TNF-α expression in the rat lung 2 weeks after irradiation, and significantly reduced TGF-β expression after 10 weeks. TNF-α expression remained unchanged 10 weeks post-irradiation. We also examined the histopathological features of radiation damage in the rat lung to determine whether cyclo(L-phenylalanyl-L-prolyl) had a radioprotective effect up to 10 weeks after irradiation. Histopathological analysis indicated that lung damage, including hemorrhaging into the alveolar space, changes in the alveolar and bronchial epithelium and blood vessels, and perivascular edema, was more severe in the radiation-alone group at 2 weeks post-irradiation than in the combination group that also received cyclo(L-phenylalanyl-L-prolyl). Specifically, we found parenchymal consolidation and alveolar epithelial edema due to focal hemorrhaging in the lungs of the radiation-alone group 2 weeks after irradiation. In contrast, the combination treatment group showed some evidence of repair of the alveolar septum and epithelial cell injury. Ten weeks post-irradiation, the combination treatment group showed less severe damage than at 2 weeks, even though this group exhibited markedly decreased damage in the alveolar space due to hemorrhaging, changes in the alveolar and bronchial epithelium and blood vessels, and perivascular edema. Two weeks after irradiation, TNF-α and TGF-β1 expressions in the alveolar epithelium, lymphoid tissues, and alveolar macrophages of the combination treatment group were markedly decreased, compared with the radiation-alone group.

Our results suggest that the use of cyclo(L-phenylalanyl-L-prolyl) would protect the lung from early damage. In addition, the utility of this compound in protection from environmental radiation damage as well as cancer radiation therapy would potentially eliminate or reduce overall side effects caused by radiation.

In summary, we demonstrated that early lung damage induced by irradiation was reduced by treatment with cyclo(L-phenylalanyl-L-prolyl) in the latent and early pneumonitis phases. Expressions of TNF-α and TGF-β1 at 2 weeks and TGF-β1 at 8 weeks post-irradiation were decreased, when animals were treated with both radiation and cyclo(L-phenylalanyl-L-prolyl), compared with animals that received radiation alone. Based on these results, we conclude that the proinflammatory cytokine TNF-α and the fibrogenic cytokine TGF-β1 are quite likely to play a role in the radioprotective effect of cyclo(L-phenylalanyl-L-prolyl). However, besides TNF-α and TGF-β1 expressions

and the reduction of free radical, the precise mechanism by which cyclo(L-phenylalanyl-L-prolyl) ameliorates the damage induced by radiation is not clear. Therefore, further studies, using electron microscopy and comparative analysis using other known radioprotective agents, are needed to develop cyclo(L-phenylalanyl-L-prolyl) as a potential radioprotective agent.

## Acknowledgment

This work was supported by a research grant from the KOSEF (Korea Science and Engineering Foundation) National Nuclear R&D Program.

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