Anti-inflammatory effect of the taffy *mu yeot*, made from the Korean radish *Raphanus sativus* L. in a lipopolysaccharide-induced murine model of pulmonary inflammation

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**Background:** Korean radish (*Raphanus sativus* L.) is used in *kimchi*, a staple of Korean cuisine. A taffy, *mu yeot*, that includes mainly radish together with other herbs (Bulbus Allii Sativi, Fructus Zizyphi, Semen Zizyphi, Rhizoma Zingiberis, and Radix Glycyrrhizae) is used traditionally in Korea to improve pulmonary symptoms. However, to our knowledge, these effects of the taffy have not yet been studied systematically.

**Objectives:** To elucidate the anti-inflammatory effects of radish taffy on macrophages and a murine model of pulmonary inflammation, and to suggest the most likely candidate for the effects.

**Methods:** A macrophage cell line, RAW264.7, was used to examine effects of the radish taffy in vitro. Pulmonary inflammation was assessed 24 h after oral instillation of lipopolysaccharide in mice treated with radish taffy for 10 days. We determined the chemical components of radish taffy quantitatively using tandem HPLC mass spectroscopy.

**Results:** Radish taffy had no apparent effect on the RAW264.7 cells. Treatment of the mice with radish taffy significantly reduced the recruitment of neutrophils to the lung, and the level of myeloperoxidase in bronchoalveolar lavage fluid (BALF). ELISAs showed that the treatment significantly decreased the level of IL 6 in BALF induced by LPS, but not the levels of IFNγ, TNFα, IL 10, or IL 12. Nor did the taffy change the levels of those cytokines or NF-κB activation in lung homogenates. HPLC-MS suggested glycyrrhizin as the most likely candidate for the anti-inflammatory effects.

**Conclusions:** Radish taffy suppresses neutrophil recruitment to lungs of mice, possibly by reducing IL 6 levels, which may lead to reduced pulmonary inflammation.

**Keywords:** IL 6, lipopolysaccharide, neutrophil, pulmonary inflammation, radish taffy

The Korean radish or *kirummuu* (*Raphanus sativus* L., also known as *Raphanus raphanistrum* L. subsp. *sativus* (L.) Domin) is a cruciferous root vegetable that is widely cultivated is a popular vegetable in Asian countries. In Korea, radish taproots are eaten raw, pickled, boiled with rice, and fermented with other seasonings as *kimchi*, a staple of Korean cuisine.

Korean radish has long been considered to aid digestion in folk medicine [1]. This suggested effect is thought to be primarily the result of multiple enzymatic activities including that of starch-hydrolyzing amylase [2], although existence of other active components is possible. For example, ingredients isolated from radish roots stimulate small bowel motility through the activation of acetylcholine receptors [3] and muscarinic receptors [4]. Moreover, radish components can stimulate the growth of lactic acid bacteria [5].

Research conducted on Korean radish has been focused on its role as a potential antitumor agent [6]. Radish extracts or its components show cytotoxic effects in vitro [7] and showed cytostatic activity and inhibited the colonization of lungs by a tumor cell line in vivo [8]. More recently, reduced expression of ErbB2 and ErbB3, members of the epidermal growth factor receptor family [9], and modulation of genes involved in the apoptotic signaling pathway [10], have been implicated as possible molecular mechanisms for the potential anticancer activities of radish extracts.

Korean radish has shown protective effects against oxidative stress, mutagens, and toxins [11, 12].

Korean radish has been traditionally used in Korea as an expectorant, to stop coughing, and to treat asthma [1]. Various parts of the radish, including the seeds, roots, shoots, and leaves, are considered to have various medicinal properties. In particular, the well-known Raphani Semen, or seeds, have long been used in Korean traditional medicine and their anti-inflammatory effects have been investigated in various studies including a bioassay-guided fractionation of a methanolic extract identifying some phenylpropanoid sucrosides as contributing to their anti-inflammatory effects [15]. However, to our knowledge, the anti-inflammatory effects of the radish root have never been studied experimentally, with the exception of a related plant, muu, the black radish root (Raphanus sativus L. var niger), a granule form of which was shown to be effective as an anti-inflammatory agent in colon mucosa of rats fed a fat-rich diet [16].

Radish taffy (“mu yeot” in Korean), commercially available as a herbal medicine in Korea, has been used as an expectorant in traditional medicine [17]. It is prepared using radish root as the major component, and 5 other herbs including Bulbus Allii Sativi (manul, or the garlic Allium sativum L.), Fructus Zizyphi (moettaechunamu, or dried ripe fruit of the Chinese date Zizyphus jujuba Mill. var jujuba), Semen Zizyphi (seeds), Rhizoma Zingberis (saeyang, or dried roots of the ginger Zingiber officinale Rosc.), and Radix Glycyrrhizae (kamcho, or dried roots and rhizomes of the licorice Glycyrrhiza inflata Batal.). In the present study, we sought to assess the anti-inflammatory effect of the taffy on the respiratory system using a murine model of pulmonary inflammation induced by lipopolysaccharide (LPS), and a macrophage cell line [18].

Methods
Preparation of RT

Six medicinal herbs, including radish (Raphanus sativus L., voucher specimen No. 150201), Bulbus Allii Sativi (Allium sativum L., voucher specimen No. 150202), Fructus Zizyphi (Ziziphus jujuba Mill., voucher specimen No. 150203), Semen Zizyphi (Ziziphus jujuba Mill., voucher specimen No. 150204), Rhizoma Zingberis (Zingiber officinale Rosc., voucher specimen No. 150205), and Radix Glycyrrhizae (Glycyrrhiza inflata Batal., voucher specimen No. 150206) were collected from a local vendor in Kyeongnam Province (southeastern Korea) from October to December, 2014. Plant materials were authenticated by Prof. Won-Hong Woo, of the Department of Herbal Resources, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan, Korea, where the voucher specimens were deposited.

Radish (900 g), Bulbus Allii Sativi (50 g), Fructus Zizyphi (50 g), Semen Zizyphi (50 g), Rhizoma Zingberis (50 g), and Radix Glycyrrhizae (30 g) were immersed in 1 L distilled water and boiled for 5 h. Brown sugar (10 g) and ground malt (45 g) was added to this mixture and after incubation at room temperature for about 14 h and this was boiled again for further 8 h. This procedure yields approximately 75 g of radish taffy.

Assay for anti-inflammatory effect in vitro

RAW264.7 cells were cultured at 37°C in a 5% CO₂ humidified incubator and maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Life Technologies) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (HyClone), 1.5 mM L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin (Sigma-Aldrich). RAW264.7 cells (1 × 10⁶ cells/mL) were stimulated with 1 mg/mL LPS in the presence of radish taffy or an equivalent volume of phosphate-buffered saline (PBS) in 48-well culture plates (Nunc). After 20 h, culture supernatants were harvested and used for a cytotoxicity assay. Phenethyl isothiocyanate (PEITC, Sigma-Aldrich), RAW264.7 cells (1 × 10⁶ cells/mL) were stimulated with 1 mg/mL LPS and diluted in dimethylsulfoxide and dilutions in PBS.

To assay cytotoxicity in vitro, 10 μL of reagent from a Cell Counting Kit (CCK)-8 (Dojindo Molecular Technologies) was added to the cultures for the final 1 h of incubation and absorbance was measured at 450 nm using a Sunrise microplate spectrophotometer (Tecan).

The anti-inflammatory effect of radish taffy in vitro was determined by the inhibition of NO release from RAW264.7 cells. NO production was assessed via measurement of nitrite in culture supernatants by a Griess reaction adapted for microplates [19]. In brief, 50 μL of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine [1:1] in 2.5% orthophosphoric acid) (Sigma-Aldrich) and 50 μL of sample was added to culture supernatants in a 96-well plate well. After 10 minutes, the absorbance of
the mixture in the plates was measured at 540 nm and nitrite concentrations were calculated based on a standard curve from a prepared standard solution of 0–100 μM sodium nitrite.

**Murine model of pulmonary inflammation and radish taffy treatment**

BALB/c mice weighing 23 ± 2 g were sourced from the Orient Co. (Seoul, Korea) and housed under constant conditions of 25 ± 1°C, 45 ± 5% humidity, under a 12:12 h light-dark cycle. The animals had free access to food pellets and sterile tap water. All animal experimental procedures used in this study were approved by Institutional Animal Care and Use Committee of Jeonju University (approval No. JU-IACUC-2015-01).

Mice received orally 100 μL of the radish taffy or equal volume of sterilized distilled water as a vehicle for 10 consecutive days. To determine an optimal dose, a quantity less than 2 g/kg was given because 2 g/kg was the maximum dose that could be orally administered in a liquid state using a gavage needle.

One hour after the final administration of the taffy, pulmonary inflammation was induced with LPS (Escherichia coli serotype B5:055; Sigma-Aldrich). To instill the LPS, mice were anesthetized with 2,2,2-tribromoethanol (Avertin) 0.3 to 0.4 g/kg body weight by intraperitoneal injection in PBS and then 10 μg of LPS in 20 μL PBS was instilled intranasally.

**Assay of anti-inflammatory effect in vivo**

Anesthetized mice were humanely killed by cardiac puncture under anesthesia and their tracheas were cannulated for bronchoalveolar lavage [20] with 4 aliquots of 0.4 mL ice-cold PBS, which were pooled to a total recovered lung lavage fluid of 1.2–1.4 mL. Leukocytes from bronchoalveolar lavage fluid (BALF) were quantified using a hemacytometer and differential cell counts for polymorphonuclear leukocytes (PMN) and mononuclear cells (MNC) were performed by counting >250 cells on cytospin preparations stained with Diff-Quik (International Reagents, Kobe, Japan). Total cell counts and calculated PMN and MNC counts are shown as cells per mL. Remaining BALF was centrifuged at 4°C at 1,000 rpm, and the supernatants were stored at −80°C until further analysis.

After BAL and flushing the lung blood vessels, the lungs were removed from the thorax, blotted with gauze to remove any remaining blood, and frozen at −20°C until assay. Lung tissue homogenates were generated as described previously [21] with slight modification. In brief, lungs were exposed to lysis buffer (0.5% Triton X-100, 150 mM NaCl, and 15 mM Tris-base; 4 mL/g tissue) containing 17 μg/mL phenylmethylsulfonyl fluoride for 30 min at 4°C, then homogenized for 30 s on ice using a polytron-type homogenizer (IKA T-10 Basic Ultra-Turrax). After centrifugation, supernatants were collected to assay for myeloperoxidase (MPO) using a DuoSet enzymelinked immunosorbent assay (ELISA) kits (R&D Systems). Absorbance was read at 450 nm on a Sunrise microplate-reading spectrophotometer.

ELISA kits were also used to measure cytokines in BALF and lung homogenate. Levels of interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL) 6, IL 10, and IL 12 were determined using commercially available ELISA kits (R&D Systems) or ELISA Opti-EIA sets (BD-Pharmingen) according to the manufacturers’ instructions.

To detect nuclear factor (NF)-κB activation, an ELISA-based assay was performed according to the recommendations of the manufacturer. In brief, frozen lungs were thawed and homogenized in 50 μl hypotonic buffer (10 mM HEPES-KCl, 1 mM β-mercaptoethanol, and 1 mM dithiothreitol). After incubation on ice for 10 min, the homogenate was vortexed for 10 s and centrifuged. The supernatant was discarded, and the pellet was resuspended in 100 μl lysis buffer in the presence of protease inhibitors, and was incubated on ice for 10 min. Cell lysates were centrifuged at 4°C and supernatants stored at −80°C until analyzed using a PathScan total NF-κB p65 Sandwich ELISA Kit or PathScan phospho-NF-κB p65 (Ser536) Sandwich ELISA Kit (Cell Signaling Technology) according to the manufacturer’s protocol.

**Quantitative analysis of anti-inflammatory components**

Tandem high-performance liquid chromatography–mass spectrometry (HPLC/MS) to determine allicin, glycyrrhizin, and gingerol was performed on a 1200 series HPLC system (Agilent Technologies). The analytical HPLC column was a Phenomenex Kinetex C18 (2.1 mm diameter × 50 mm, 2.6 μm particle size) and the sample injection volume was 3 μL. The column was eluted with a gradient of water in acetonitrile containing 0.1% formic acid at 0.5 mL/min. Mass spectrometric detection was with an Agilent...
6410B instrument. Ions were generated in positive ionization mode using electrospray ionization interface. The fragmentor potential was 110 V and the interface heater was at 300°C. We identified PEITC by gas chromatography using a mass selective detector (Shimadzu GCMS-QP2010 Ultra), equipped with Labsolution software and National Institute of Standards and Technology (NIST) spectral data. A DB-5MS fused silica capillary column (0.25 mm bore × 30 m, 0.25 μm film thickness) was used for separations. The chromatographic conditions were as follows: column temperature 70°C (3 min), 70–300°C (10°C/min), 300°C (5 min), injector temperature 250°C, split ratio 1:30, carrier gas helium, flow rate 1.0 mL/min, ionization energy 70 eV, mass range 50–550, volume injected 1 μL.

Statistical analyses
Data are presented as mean ± standard error of the mean (SEM). All representative experiments were repeated at least three times. Statistical analysis was performed using a Student t test and one-way analysis of variance (ANOVA) followed by a post hoc Tukey test. P < 0.05 was considered significant. All statistical tests were conducted using SPSS software (version 23; IBM Corp).

Results

Anti-inflammatory effect of radish taffy in vitro
Before examination in vivo, the effect the taffy on macrophage activity was evaluated in vitro. We examined whether radish taffy can inhibit NO and proinflammatory cytokine production by RAW264.7 macrophages stimulated with LPS. As shown in Figure 1, left panel the radish taffy was strongly cytotoxic at 10 mg/mL and partially cytotoxic at 1 mg/mL (85.0% survival). NO and IL-6 production were slightly decreased by 1 mg/mL of radish taffy (data not shown), although this effect may be the result of mild cytotoxicity. We failed to find suppression of NO or cytokine production by RAW264.7 cells by radish taffy at 0.01 or 0.1 mg/mL. Rather, radish taffy at 0.1 mg/mL weakly activated macrophages to secrete NO and cytokines, but the effect was not significant. PEITC (5 μM) as a positive control strongly inhibited NO and cytokine production (Figure 1, right panel).

Figure 1. Effect of radish taffy on inflammatory activity of RAW264.7 cells in vitro. (Left panel) To examine the cytotoxicity of radish taffy, 10 μL of cell counting kit (CCK)-8 reagent in the final 1 h of incubation was added to cells exposed to 0–10 mg/ml of radish taffy and lipopolysaccharide, and then absorbance of the supernatant measured at 450 nm. (Right panel) To examine the effect of radish taffy on NO and cytokine production, cells were exposed to 0.01 or 0.1 mg/mL of radish taffy and 5 μM of PEITC for 20 h and then NO and cytokine levels in the supernatants were measured. Accumulated data from 2 separate experiments with similar results are shown and data are presented as means ± standard error of the mean. **P < 0.01 (n = 8).
Effect of radish taffy on LPS-induced pulmonary inflammation in vivo

Intranasal instillation of LPS in mice triggers an acute pulmonary inflammation, characterized by increases in total leukocyte numbers in BALF. In our experiment, leukocyte number in the control group was increased around 4.4-fold by LPS instillation (4 mice/group). However, the numbers in the radish taffy-treated groups were reduced in a dose-dependent manner (Figure 2).

In the following experiments, vehicle and treated groups received sterilized distilled water or the highest dose of 2.0 g/kg of radish taffy, respectively, and accumulated data are shown in Figure 2. Total leukocyte number in treated group was markedly lower than vehicle (Figure 3A). PMN number also significantly decreased (Figure 3B), whereas the numbers of MNC between vehicle and treated groups were comparable (Figure 3C) and other mononuclear cells were scarcely observed.

Figure 2. Dose–response effect of radish taffy (RT) on recruitment of leukocytes to airways in mice with induced pulmonary inflammation. Mice received 0.2–2 g/kg of RT (100 μL) orally or an equal volume of sterilized distilled water as a control for 10 days. One hour after the last administration of RT, pulmonary inflammation was induced by instillation of lipopolysaccharide (LPS 10 μg) and bronchoalveolar lavage fluid was analyzed 24 h later. Accumulated data from 2 separate experiments with similar results are shown. **P < 0.01 (n = 8).

Figure 3. Effect of radish taffy (RT) on the cellularity in bronchoalveolar lavage fluid (BALF). (A) Total leukocyte numbers in BALF were counted using a hemacytometer (***P < 0.01, n = 10) and (B) the numbers of polymorphonuclear leukocytes (PMN), and (C) mononuclear cells (MNC) were calculated with cytospin preparations stained with Diff-Quik. **P < 0.01 (n = 10).
MPO ELISA has been used as a measure of PMN accumulation in the lung [22]. In the present study, MPO levels were assessed by ELISA in BALF and homogenized lung tissue. As shown in Figure 4, MPO level in BALF of treated group was markedly lower than that in the vehicle group. Similarly, its level in the lung was significantly suppressed by radish taffy treatment.

**Effect of radish taffy on cytokine secretion in vivo**

To gain insight into the mechanism underlying radish taffy-mediated suppression of pulmonary inflammation, cytokine levels in BALF and lungs were examined. The level of the proinflammatory cytokine, IL 6 in BALF of treated group was significantly lower than that in vehicle group, whereas levels of TNFα and IFNγ were unaffected by radish taffy treatment (Figure 5, left panel). The level of IL 10, an anti-inflammatory cytokine, was not affected by radish taffy treatment, and there was no apparent difference in the level of IL 12, which is produced by activated macrophages. Levels of cytokines appeared much higher in the lung homogenates, although there were no significant differences in any cytokine level between groups (Figure 5, right panel).

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**Figure 4.** Effect of radish taffy (RT) on myeloperoxidase (MPO) expression in bronchoalveolar lavage fluid (BALF) and lung tissue. BALF and lung homogenates were prepared and MPO levels were determined using an ELISA. **P < 0.01 (n = 10).**

**Figure 5.** Effect of radish taffy (RT) on cytokine secretion. Levels of interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL) 6, IL 10, and IL 12 were determined with ELISAs in bronchoalveolar lavage fluid (BALF) (left panel) and lung homogenates (right panel). **P < 0.01 (n = 14).
Effect of radish taffy on NF-κB activation in vivo

NF-κB is a key transcription factor for many proinflammatory cytokine genes, and thus affects production of these proteins, which in turn trigger inflammation. Activation of NF-κB in airway epithelium is sufficient to induce neutrophilic lung inflammation [23]. The NF-κB binding site is responsible for controlling IL-6 production [20]. We thus investigated the effect of RT treatment on NF-κB activation in the lung tissue. Total and phosphorylated NF-κB p65 (Ser\(^{536}\)) levels were determined and data shown in Figure 6 suggests that treatment with radish taffy does not reduce NF-κB activation.

Radish taffy content of several compounds known as anti-inflammatory

Four substances in radish taffy, namely allicin, glycyrrhizin, gingerol, and phenethyl isothiocyanate (PEITC) were selected as putatively active components and their content was quantitated with HPLC/MS and GC/MS. Among these compounds, the most abundant was glycyrrhizin (2.38 mg/g) as shown in Figure 7B and Table 1, allicin and gingerol content were low (0.1 to 2.0 \(\mu\)g/g) (Figure 7A, 7C and Table 1), while PEITC was undetectable (Figure 8).

Discussion

For the first time to our knowledge, evidence for a possible mechanism of action of the folk remedy radish taffy is presented. We did not find an inhibitory effect of radish taffy on NO or cytokine secretion by the RAW264.7 macrophage cell line at sub-cytotoxic concentrations in vitro. These findings suggest that RT does not have a direct anti-inflammatory effect.

In vivo, total leukocyte and PMN numbers in BALF were significantly lowered by oral administration of radish taffy in a murine model of pulmonary inflammation induced by LPS. Moreover, MPO levels in BALF and lung tissue were markedly reduced by pretreatment with radish taffy. MPO is found in azurophilic granules of neutrophils [24] and ELISA for MPO has been used as a measure of PMN accumulation in the lung [22]. The reduced MPO levels we found suggest that radish taffy reduces pulmonary inflammation induced by LPS. However, we did not examine this histologically in the present study. Nevertheless, it is widely accepted that neutrophil accumulation in airways results from their migration from lung tissue [25], and the data from our BALF analysis show typical features found in the model of pulmonary inflammation. The appearance of mononuclear cell types other than macrophages was scant. Thus, our data likely reflect infiltration of neutrophils from lung tissue.

![Figure 6](image-url)
Figure 7. Total ion chromatograms of allicin (A), glycyrrhizin (B) and gingerol (C) for each standard (upper panels) and radish taffy sample (lower panels) using liquid chromatography–mass spectrometry (HPLC/MS) on a 1200 series HPLC system (Agilent Technologies) using a reversed phase column in the acquisition condition. Mass spectrometric detection was performed using Agilent 6410B instrument and ions were generated in positive ionization mode using electrospray ionization interface (intensity vertical axis). The fragmentor potential was 110 V and the interface heater 300 °C.
Although accounting for discrepancy between in vitro and in vivo results is complicated, it is presumed that the components of radish taffy are not directly effective and need to be metabolized to have an anti-inflammatory effect. It is also possible that metabolites from radish taffy components can act differentially and interact synergistically in vivo. Moreover, using a cell line adapted to in vitro culture to evaluate the effect may have limitations.

A limitation of the present study was that we could not administer radish taffy at doses of more than 2 g/kg because this was the maximum practical dose that could be orally administrated in a liquid state using a gavage needle. Given that dose of 2 g/kg was most effective, it is possible that a stronger effect might be observed with higher doses.

Table 1. Quantification of 4 anti-inflammatory compounds in radish taffy by HPLC/MS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time</th>
<th>Sample peak area*</th>
<th>Content in sample (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allicin</td>
<td>4.58</td>
<td>313</td>
<td>0.1</td>
</tr>
<tr>
<td>Glycyrrhizin</td>
<td>6.28</td>
<td>768</td>
<td>2,380</td>
</tr>
<tr>
<td>6-Gingerol</td>
<td>6.56</td>
<td>2886</td>
<td>2.0</td>
</tr>
<tr>
<td>Phenethyl isothiocyanate</td>
<td>13.87</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Sample was diluted to 40 mg/mL in distilled water and injection volume was 3 μL. ND = not detected.

Figure 8. Total ion chromatogram of standard phenethyl isothiocyanate (PEITC) (A) and radish taffy sample (B) using gas chromatography–mass spectrometry (GC/MS). Gas chromatography was performed on a DB-5MS fused-silica capillary column using a mass selective detector (Shimadzu GCMS-QP2010 Ultra) equipped with Labsolution software and National Institute of Standards and Technology spectra data.
Inflammation is governed by a complex network of cytokines [26]. In an inflammatory response following infection, activated macrophages release a variety of cytokines and other factors to modulate tissue damage and repair. We hypothesized that the taffy-mediated reduction of pulmonary inflammation may be a result of decreased production of proinflammatory cytokines or elevated production of anti-inflammatory cytokines. Radish taffy did not change the level of TNFα, a critical cytokine in inflammatory response, and no changes were observed in the levels of IFNγ or IL 12, which might largely originate from macrophages. Nor did the taffy affect the production of IL 10, an anti-inflammatory cytokine that downregulates the production of proinflammatory cytokines [27], suggesting that IL 10 is not involved in the anti-inflammatory effect of radish taffy. RT did not reduce NF-κB activation in the lung. Although NF-κB pathway is critical in IL 6 signaling, IL-6 expression is also controlled by mitogen-activated protein (MAP) kinases [28] and other regulators such as ANXA1 [29] and MAP kinase phosphatase-1 (MKP-1) [30]. Further study will be required to determine which is the target for the active components of radish taffy or its metabolites.

Our data showed that radish taffy treatment significantly inhibited IL 6 secretion. IL 6 is a central regulator of immune responses in pneumonia and IL 6 concentrations in blood and BALF of patients suffering from pneumonia are associated with disease severity [31]. Results from studies of IL 6-deficient mice indicate a prominent role of the cytokine IL 6 in pneumonia [32]. Moreover, IL 6 is essential in airway recruitment of neutrophils [33]. Our data suggest that radish taffy suppresses IL 6 secretion and thereby reduces airway neutrophilia.

It is curious that RT inhibits IL 6, but not TNFα production. IL 6 and TNFα release by macrophages can be differentially regulated depending on environmental factors [34]. It may be that the taffy does not influence the activity of macrophages, but instead other IL 6-producing cells such as fibroblasts and endothelial cells. This hypothesis is consistent with our results in that radish taffy did not alter the production of IL 12 or TNFα, which are produced largely by macrophages. Identification of the main cellular targets of radish taffy in vivo is critical for understanding the mechanisms of the anti-inflammatory action of radish taffy.

The putatively active components of radish taffy were examined. Although the taffy is comprised of a number of components, we selected several compounds already known for their anti-inflammatory effect for quantification. As the main herb, radish contains the anti-inflammatory ingredient, PEITC, which inhibits inflammatory reactions by two distinct pathways in mast cells in vitro [35]. Another candidate is 1,2-vinylidithin, a organosulfur also found in garlic, formed in the breakdown of allicin, which inhibits human preadipocyte differentiation and has anti-inflammatory properties [36]. Glycyrrhizin, a component of Radix Glycyrrhizae, and 6-gingerol in Rhizoma Zingiberis, exert anti-inflammatory effects [37, 38]. Table 1 shows that glycyrrhizin has the highest concentration in radish taffy, and allicin, and 6-gingerol are found in much lower levels, while PEITC was not detected. Although radish contains PEITC, it was not surprising that PEITC was not detectable in radish taffy because this molecule is highly volatile. Our data indicate that PEITC was lost during the preparation of the taffy. This loss of PEITC may account for the lack of any substantial anti-inflammatory effect of the taffy in vitro. One of the major components of radish taffy, that could account for its anti-inflammatory effect in vivo is glycyrrhizin. However, a limitation of our study is that analysis of all components of all 6 medicinal herbs was not possible. It is likely that there are other undefined components with anti-inflammatory effects and that they may interact synergistically. Further research such as activity-guided fractionation will be required to identify active components. Recently, phenylpropanoid sucrosides exerting anti-inflammatory activity were identified as anti-inflammatory components of a methanolic extract of Raphanus sativus seeds [15]. It will be interesting to determine whether these compounds also exist in the radish root.

Despite improved treatment, acute lung injury is a common disease with high incidence and mortality worldwide. Damage as a result of inflammation may occur in the alveolar capillary and epithelial membranes by infiltration of neutrophils and monocytes, which can lead to leakage of proteinaceous edema fluid into the alveolar space and impaired gas exchange [39]. Unfortunately, current pharmacological treatments are only marginally effective [39]. Moreover, other lung diseases, such as severe asthma, chronic obstructive pulmonary disease, cystic fibrosis, bronchiectasis and infiltrative pulmonary diseases are associated with chronic neutrophilic inflammation [40]. Our data demonstrating that radish taffy may reduce neutrophil recruitment to lung tissue suggests a
potential role for radish taffy in complementary medicine to prevent or attenuate pathophysiological consequences of these lung disorders.

Conclusions
Radish taffy failed to show a substantial anti-inflammatory effect on RAW264.7 cells at sub-cytotoxic concentrations in vitro. However, in vivo, radish taffy reduced airway neutrophilia and MPO levels in a model of pulmonary inflammation induced in mice by LPS, and this effect may result from observed suppression of IL 6 release into the airway fluid. Thus, radish taffy has a therapeutic potential for the treatment of neutrophil-driven inflammatory diseases.

Author contributions
S-Y Nam and D-H Kim conceived and designed the study, collected, analyzed, and interpreted the data. D-H Kim drafted the manuscript and S-Y Nam critically revised it. Both authors approved the final submitted version and take responsibility for the statements in the published version.

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Conflict of interest statement
The authors have no conflicts of interest to declare.

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