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ORIGINAL ARTICLE

Lung injury induced by the pulmonary instillation of povidone-iodine in rats

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Abstract
Purpose Povidone-iodine (polyvinylpyrrolidone iodine, PI), which is commonly used as a pre- and postoperative oral antiseptic, has been reported to cause pneumonia secondary to its pulmonary aspiration. Because no studies have yet investigated the underlying mechanisms of PI-induced pneumonia, we conducted an animal study to analyze the effect of PI on the lung following its pulmonary instillation.
Methods The lungs of 61 male Sprague–Dawley rats (150–250 g) were instilled with varying volumes of either phosphate-buffered saline or PI solutions varying in strength from 0.01% to 10%. The lungs were harvested from the rats 1 h or 1, 3, 5, 7, 14, or 21 days after instillation for radiologic examination, macroscopic and light and scanning electron microscopic assessment, and an assessment of pulmonary toxicity using an MTT-based cytotoxicity assay.
Results Macroscopically, atelectasis was the primary pulmonary lesion after PI instillation. The primary light and scanning electron microscopic findings were an initial inflammatory phase with edema, alveolar rupture, and leukocyte infiltration into the pulmonary interstitium, which progressed into a phase of lung parenchyma loss, and then resolved itself with scar tissue formation. Lung tissue viability following 1-day exposure to 0.01%, 0.1%, 1%, or 5% PI progressively decreased in a significant dose-dependent manner.
Conclusions PI aspiration can cause lung injury, including pulmonary fibrosis.

Keywords Lung injury · Povidone-iodine · Pulmonary instillation

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Introduction

Povidone-iodine (polyvinylpyrrolidone iodine, PI), which is a broad-spectrum microbicide with activity against bacteria, viruses, fungi, and protozoans [1, 2], is commonly used as a pre- and postoperative antiseptic and a disinfectant of skin wounds. PI is also used as a gargle in various concentrations to (a) disinfect the oral cavity before oral surgery [3], (b) minimize the likelihood of developing respiratory tract infections in mechanically ventilated patients [4–6], and (c) forestall the common cold and influenza [7]. When used as an oral disinfectant in anesthetized patients, pulmonary aspiration of the oral PI solutions rarely occurs even when the inflated cuff of the endotracheal tube does not provide a complete seal. However, there are two published case reports of pneumonia secondary to the pulmonary aspiration of an oral PI solution used as an oral antiseptic before surgery under general anesthesia [8, 9]. Because no studies on the underlying mechanisms of PI-induced pneumonia have yet been undertaken, we conducted an animal study to analyze the effect of PI on the lung following its pulmonary instillation.

Materials and methods

The study, the use of animals, and all procedures that the animals underwent were reviewed and approved by our Institutional Animal Care and Use Committee before the start of the study.

Materials

Sixty-one male Sprague–Dawley rats (150–250 g), which were free of known respiratory pathogens, were used in the study. The rats were purchased from Charles River Laboratories, Seoul, Korea, and were allowed at least 1 week of acclimatization in the Animal Care Facility in our institution before use. The rats were housed in plastic cages (two rats per cage) under controlled conditions: 22° ± 2°C ambient temperature; 50% relative humidity; and a light:-dark cycle of 12:12 h with lights on at 0600 hours. The rats had free access to tap water and a pelleted rat chow (Cargill Agri Purina, Seongnam, Gyeonggi, Korea), which contained 20% crude protein, 4.5% crude fat, 6% crude fiber, and 12.15 MJ/kg net energy.

Methods

Anesthesia and experimental procedures

Each rat was anesthetized by a 5% enflurane-oxygen mixture (1 l/min), and then intubated endotracheally with a 16 G venous catheter using a previously published method [10]. Once anesthetized, the rats were monitored by observing the respiratory pattern (rate) and the color of the nose and extremities. A PI solution of varying strengths (see later) was instilled into the lungs of the anesthetized rats through a closed-end epidural catheter (BD Perisafe; BD Medical Systems, Erembodegem, Belgium), which was passed through the endotracheal catheter using a previously described method [11, 12]. After PI instillation, the epidural catheter and the endotracheal tube were removed, and the rat was allowed to recover from anesthesia. The duration of anesthesia and pulmonary PI instillation in each rat was approximately 5 min. At various times after the pulmonary PI instillation, the lungs were examined radiologically or harvested for macroscopic or microscopic assessment and assessment of the pulmonary toxicity of PI using an MTT-based cytotoxicity assay (see later). Figure 1 is a flow chart summarizing the experimental procedures and the number of rats that were used for each procedure.

Preliminary experiments

As there is no published information on the toxic dose of PI to the lung, we performed preliminary experiments to determine the lethal, survival, and lung injury-inducing doses of PI. For this purpose, the various PI solutions were prepared by diluting PI in phosphate-buffered saline (PBS) (Fig. 1).

(a) Determination of the lethal PI dose Five rats (one rat/treatment) were used to determine the lethal dose of 0.5 ml of a PI solution of varying strengths (10%, 5%, 1%, and 0.1%) that was instilled into the lungs. For this purpose, 0.5 ml PI solution or 0.5 ml PBS (control) was instilled into the lungs of an anesthetized rat through the intubated venous catheter without insertion of epidural catheter. The two rats whose lungs were instilled with 0.5 ml 5% and 10% PI died immediately after the instillation without recovering from anesthesia. The rats whose lungs were instilled with 0.5 ml PBS and 0.5 ml 0.1% and 1% PI recovered from anesthesia.

(b) Determination of the survival PI dose In the light of the results of the experiment that determined the lethal PI dose, five rats (one rat/PI dose) were then used to determine the survival dose of a 5% PI solution after its pulmonary instillation. For this purpose, 0.5, 0.4, 0.3, 0.2, or 0.1 ml 5% PI solution was instilled into the lungs of an anesthetized rat through the intubated venous catheter without insertion of an epidural catheter. The rats that received either 0.1 or 0.2 ml of the 5% PI solution recovered from anesthesia: the remaining three rats died immediately following the pulmonary PI instillation without recovering from anesthesia.

(c) Determination of the lung injury-inducing PI dose In the light of the results of the experiment that determined the survival PI dose, we decreased the strength of the PI solution to determine the lung injury-inducing PI dose in eight rats (one rat/PI dose). The strength of the PI solution was reduced to lessen the severity and extent of the pulmonary hemorrhage and edema, which we presumed were the causes of death after the intratracheal instillation of 0.5 ml 5% PI solution. In these rats, 0.2 ml of PI solution of different strengths (0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%) was unilaterally instilled into one lung of an anesthetized rat using an epidural catheter that was placed intrabronchially by passing it through the intubated venous catheter using a previously published method [12]. The rats were killed after 21 days by an overdose of enflurane inhalation anesthesia, and the lungs were harvested for macroscopic and microscopic assessment by standard methods with hematoxylin and eosin (H&E) staining.

Assessment of lung injury induced by intrabronchial instillation of PI

In a preliminary study, the primary macroscopic and microscopic pulmonary lesion was atelectasis. Because

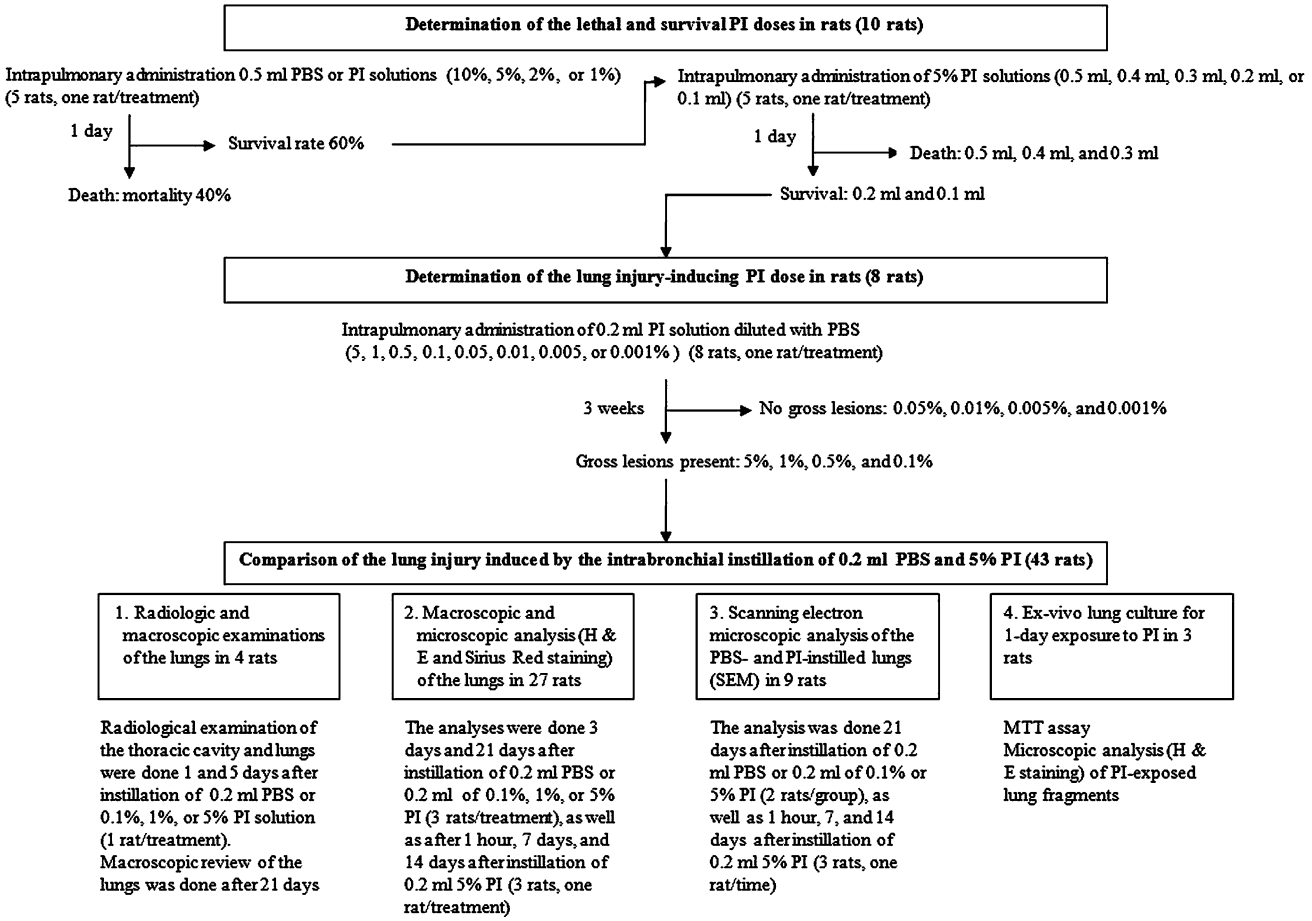


Fig. 1 Flow chart of the study. *PBS* phosphate-buffered saline, *PI* povidone-iodine, *H&E* hematoxylin and eosin, *MTT* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

these findings were consistent with those in the lungs of the rats that had been instilled with PI solutions of strength greater than 0.05% PI (photographs not shown), we decided to use 0.2 ml 0.1%, 1%, and 5% PI solution for inducing unilateral pulmonary damage to investigate PI-induced pneumonia following its intrabronchial instillation through the intubated venous catheter using a previously published method [12].

(a) Radiologic examinations (four rats) Radiologic examination of the thoracic cavity and lungs of rats (one rat/dose) was done at 1 or 5 days after an intrabronchial instillation of 0.2 ml PBS or 0.1%, 1%, or 5% PI solution. After 21 days, the rats were killed by an overdose of enflurane inhalation anesthesia, and the lungs were harvested for macroscopic assessment.

(b) Macroscopic and microscopic assessment of PI-induced lung injury (36 rats) Twenty-seven rats were used in the macroscopic and microscopic assessment of the PI-induced lung injury after intrabronchial instillation of 0.2 ml PBS or 0.1%, 1%, or 5% PI solution under enflurane anesthesia using an epidural catheter that was passed through the intubated venous catheter. Twenty-four of these rats were weighed daily before being killed 3 or 21 days after the instillation by an overdose of enflurane inhalation anesthesia. The remaining 3 rats were intrabronchially instilled with 0.2 ml 5% PI solution and then killed in the same manner at 1 h (1 rat), or on day 7 (1 rat) or day 14 (1 rat) after instillation. For the microscopic assessment, one lung of the PBS-treated rats and the damaged lung of each PI-treated rat were harvested and then fixed in 4% phosphate-buffered formalin for 24 h. After embedding in paraffin, 4-μm-thick paraffin sections of the lungs were prepared and processed for hematoxylin and eosin (H&E) staining to assess changes in the microarchitecture and Sirius Red staining to assess the extent of collagen formation/deposition under a light microscope. The lungs of nine rats were used in a scanning electron microscopic assessment of the PI-induced lung injury. For this purpose, the lungs of 6 rats (2 rats/group) were intrabronchially instilled with 0.2 ml PBS, 0.2 ml 0.1% PI, or

0.2 ml 5% PI, and then killed 21 days after the instillation by an overdose of enflurane inhalation anesthesia. The lungs of the remaining 3 rats were intrabronchially instilled with 0.2 ml 5% PI solution, and the rats were killed in the same manner at 1 h (1 rat), day 7 (1 rat), or day 14 (1 rat) after the instillation. Briefly, the injured lung of each PI-treated rat and one lung of the PBS-treated rat were first fixed in 3% glutaraldehyde for 48 h at room temperature. After fixing, a sample of injured lung tissue from the PI-treated rats and a sample of normal lung tissue from the PBS-treated rats was cut into small pieces and fixed in 2% glutaraldehyde. After overnight fixation at 4°C, lungs were sequentially soaked for 1 h in 12.5%, 25%, and 50% ice-cold dimethyl sulfoxide (DMSO), and then dehydrated in an increasing series of ethyl alcohol dilutions. Finally, the cryofractured lungs were then examined under the scanning electron microscope (SEM) (JSM-6100; JEOL, Tokyo, Japan) assess the resultant changes. Macroscopic and microscopic assessments of all lungs were done by a pathologist who was blind to the various treatments.

(c) Pulmonary cytotoxicity of PI (three rats) The three rats were killed by an overdose of enflurane inhalation anesthesia, and the lungs were immediately harvested for ex vivo lung culture. Briefly, the lungs were cut into 2- to 3-mm^3 fragments using a scalpel under sterile conditions. The lung fragments were first washed three times with PBS that contained 5 μg/ml fungizone (Gibco, Grand Island, NY, USA) and 10 μg/ml gentamicin (Gibco), and then rinsed with culture medium [99% Dulbecco's minimum essential medium (DMEM); Gibco] that contained 1% fetal calf serum (Hyclone Laboratories, Provo, UT, USA), and 10 μg/ml gentamicin. To determine the cytotoxicity of PI on the lung, the washed lung fragments were incubated in DMEM that was supplemented with 0.01%, 0.1%, 1%, or 5% PI for 1 h under constant shaking at 15 rpm in an incubator at 4°C. At the end of the incubation, the lung fragments were transferred to hydrated 1 × 1 cm gelatin sponges (Spongostan; Johnson & Johnson, San Angelo, TX, USA), which were then placed into each well of a 6-multiwell plate. A 3-ml aliquot of DMEM with no PI or supplemented with 0.01%, 0.1%, 1,% or 5% PI was then added to each well in such a manner that the lung fragments on the gelatin sponges appeared to be on a raft above the liquid phase. The plates were then placed in a humidified incubator and maintained at 37°C in 95% air/5% CO_2 under continuous shaking at 15 rpm for 24 h. The cytotoxicity of PI was assessed using an MTT-based cytotoxicity assay and a microscopic analysis of the PI-exposed lung fragments. For the MTT assay, 20-mg lung fragments were incubated for 3 h at 37°C in DMEM that contained 2 mg/ml MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide; Roche Diagnostics, Mannheim, Germany]. At the end of the incubation, the pulmonary tissues washed three times with PBS, and then treated with 1 ml DMSO at room temperature for 20 min in a shaker to solubilize the formazan crystal. The absorbance of the soluble formazan was then measured spectrophotometrically at optical density $(OD)_{570}$. Viability was determined by calculating the ratio of the OD_{570} absorbance values that were recorded before and after the exposure of the lung tissue to PI culture using the following equation: relative viability (%) = 100 × (absorbance at OD_{570} of 20 mg lung tissue after exposure to PI/absorbance at OD_{570} of 20 mg lung tissue before exposure to PI). The MTT assay was done in quadruplicate. Microscopic assessment of the PI-exposed lung fragments was identical to that described in (b) of the "Methods" section.

Statistical analysis

Data from the PI cytotoxicity (percentage viability) assay were statistically analyzed by the Kruskal–Wallis test using a computerized statistical software program (MedCalc for Windows, version 10.0; MedCalc Software, Mariakerke, Belgium). Results are expressed as mean ± standard deviation (SD), and P values < 0.05 were considered to be statistically significant.

Results

Over the 21-day study period, the body weights of the PI-instilled rats were similar to those of the PBS-instilled rats (data not shown).

Radiologic examinations

No lesions were seen in the radiographs or found after macroscopic examination of the thoracic cavity and lungs of rats whose lungs were instilled with PBS. The radiologic examination of the lungs 1 day after the intrabronchial PI instillation revealed an edematous-like lesion in the cranial lobe of the lung that was instilled with 0.1% PI (Fig. 2b1) and in the caudal lobe of lungs which were instilled with either 1% or 5% PI (Fig. 2c1, d1). In all PI-instilled lungs, these pulmonary lesions were not seen when the lungs were radiologically reexamined 5 days later (Fig. 2b2–d2). When the lungs were harvested after 21 days, pulmonary lesions were found in the PI-instilled lungs at the same locations that were seen in the 1-day radiologic examinations (Fig. 2b3–d3).

Macroscopic findings in the PI-instilled lungs

No lesions were found in the thoracic cavity and the lungs of PBS-instilled rats (Fig. 3a, e). Gross examination of the

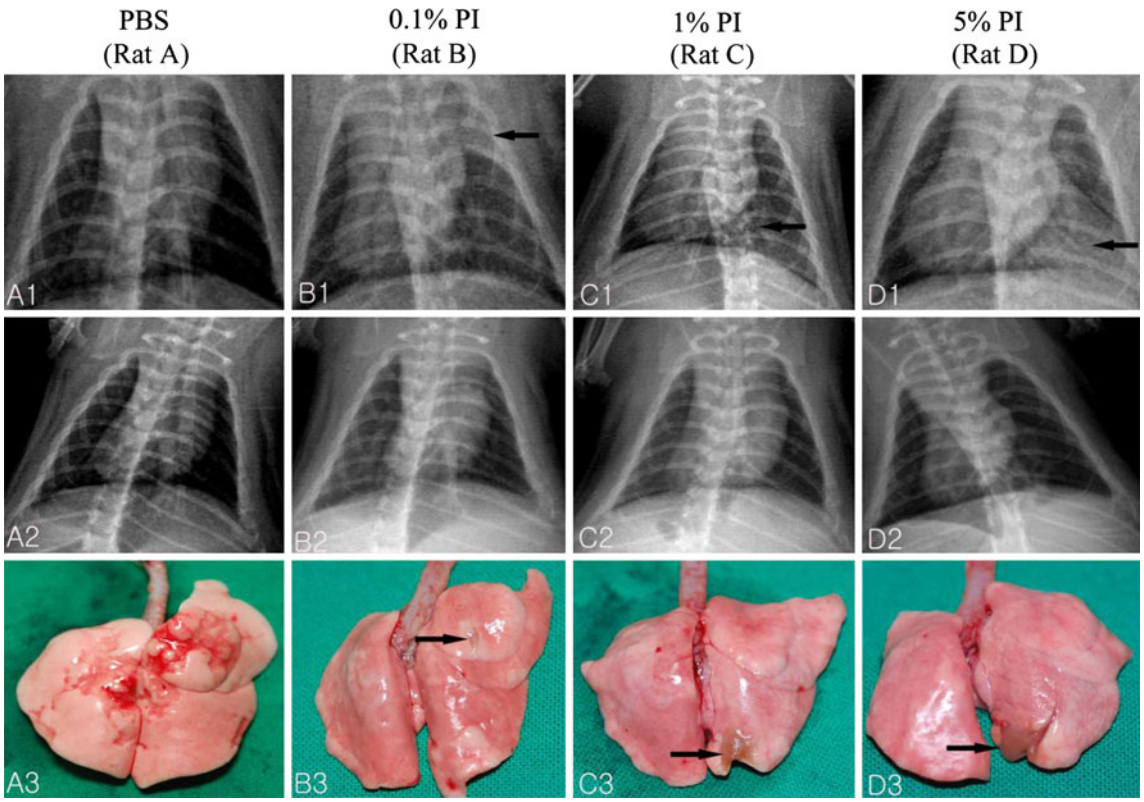


Fig. 2 Radiographs of the thoracic cavity and lungs 1 day (**a1–d1**) and 5 days (**a2–d2**), and macroscopic findings in the lung 21 days (**a3–d3**), after intrabronchial administration of 0.2 ml phosphate-buffered saline (PBS) or 0.2 ml 0.1% povidone-iodine (PI), 1% PI, and 5% PI solutions. *Arrows* in **b1**, **c1**, **d1** point to edematous-like lesions in the lungs that are suspected to have been caused by PI; *arrows* in **b3**, **c3**, **d3** point to atelectasis-like lesions suspected to have been caused by PI

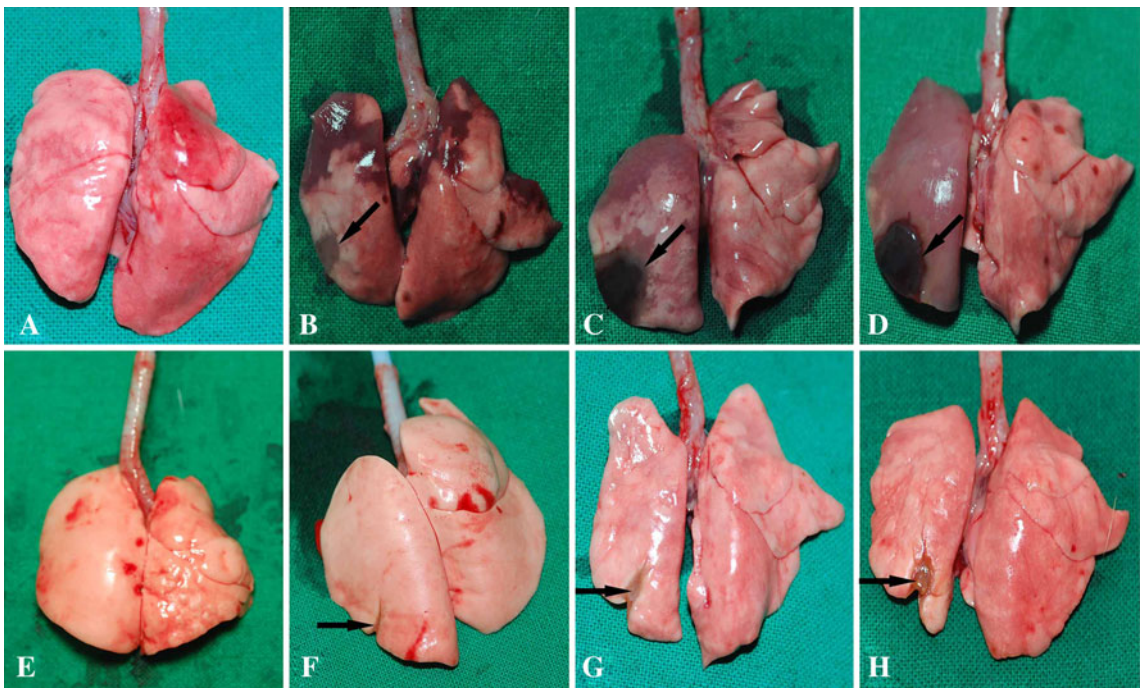


Fig. 3 Macroscopic findings in the lungs 3 days (**a–d**) and 21 days (**e–h**) after intrabronchial instillation of 0.2 ml phosphate-buffered saline (PBS) (control) (**a**, **e**), and 0.2 ml 0.1% povidone-iodine (PI) (**b**, **f**), 1% PI (**c**, **g**), and 5% PI (**d**, **h**) solutions. Lesions were found in the lower region of the PI-instilled lung at all PI concentrations (*arrows* in **b–d**, **f–h**)

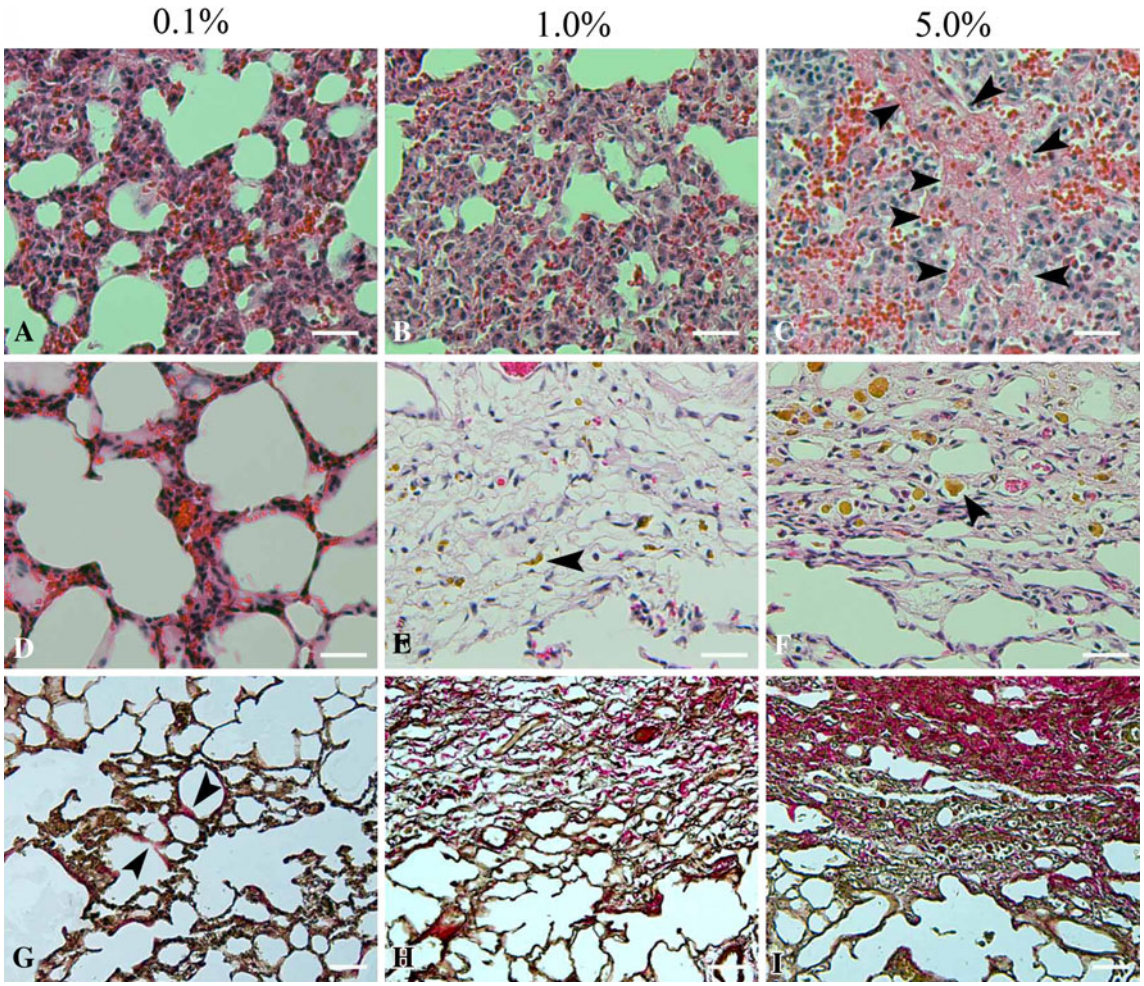


Fig. 4 Intrabronchial administration of PI induces alveolar damage (**a–c**). Intrabronchial instillation of PI induces lung fibrosis of varying degrees 21 days after its instillation (**d–i**). *Bars* 100 μm

thoracic cavity and harvested lungs of the PI-instilled rats revealed that a healing process had occurred after the intrabronchial instillation. No adhesions or evidence of leakage caused by perforations were observed in the thoracic cavity. Regions of the lungs of all rats in the three groups of PI-instilled rats were atelectatic or collapsed (Fig. 3f–h). Among the three groups of PI-instilled rats, the macroscopic appearance of the lungs in the rats whose lungs were instilled with 5% PI was similar to that in the rats whose lungs were instilled with 1% PI, but differed from that in the rats whose lungs were instilled with 0.1% PI (Fig. 3b, d, f, h).

Light microscopic findings in the PI-instilled lungs

No microscopic lesions were found in the lungs of PBS-instilled rats. Histological examination of the lungs of rats whose lungs were instilled with the lowest PI dose (0.2 ml 0.1% PI) showed ruptured alveoli with microcyst formation on day 3. Widening of the interstitium between the alveoli had occurred, and neutrophils and eosinophils had infiltrated into the interstitium (Fig. 4a). The pulmonary lesion was exacerbated in the lungs of rats whose lungs were instilled with the intermediate PI dose (0.2 ml 1% PI) on day 3: more enlarged microcysts and inflammatory cell infiltration in the interstitium were seen than that observed in the lungs of the rats whose lungs were instilled with the lowest PI dose (Fig. 4b). These changes were further exacerbated in the lungs of the rats whose lungs were instilled with the highest PI dose (0.2 ml 5% PI) on day 3: there was very evident necrosis of the lung parenchyma, such as confluent necrosis of alveoli and deposition of hyaline in alveolar spaces (Fig. 4c). Histological examination of the lungs of rats whose lungs were instilled with the lowest PI dose (0.2 ml 0.1% PI) showed interstitial fibrosis of the alveolar walls on day 21 (Fig. 4g). Multifocal alveolar rupture, interstitial pneumonitis, and fibrosis can be seen in the PI-induced lung injury. The alveolar

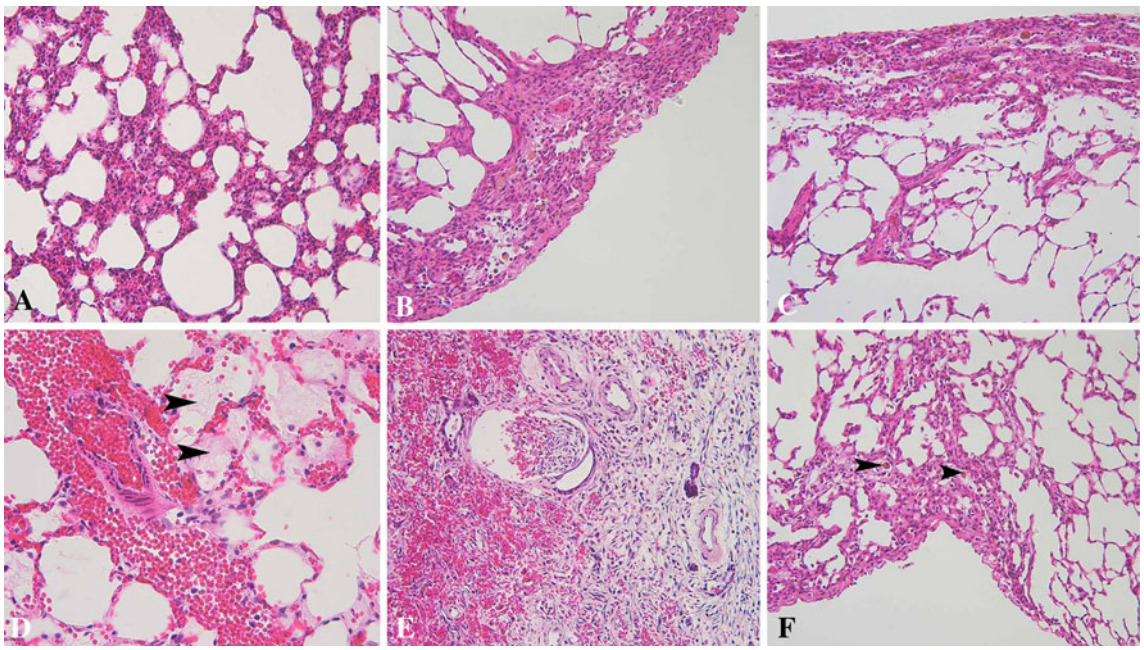


Fig. 5 Microscopic findings in the lungs 21 days after intrabronchial administration of 0.2 ml 0.1% (**a**), 1% (**b**), or 5% (**c**) povidone-iodine (PI) solution. Microscopic findings in the lung 1 h (**d**), 7 days (**e**), and 14 days (**f**) after intrabronchial instillation of 0.2 ml of % PI solution. *Arrowheads* in **d** and **f** point to PI-like pigments. Sections were stained with hematoxylin and eosin. ×200

septa were infiltrated by inflammatory cells and were widened by fibrous tissue accumulation (Fig. 5a–c). The consolidated areas in the injured lung show marked fibrosis and heavy infiltration by inflammatory cells (Fig. 5b, c). In fact, the alveoli had abnormal shapes, and some had collapsed and were torn (Fig. 5a–c). With the higher PI doses (0.2 ml 1% or 5% PI), fibrotic scar tissue had replaced necrotic lung parenchyma. In the 5% PI-instilled lung, the scar tissue was more compact and there was more deposition of collagen bundles than that found in the 1% PI-instilled lung. Within the scar tissue, PI pigment-laden monocytes were frequently observed (Fig. 4e, f). When the histological changes in the lung at the different time points were compared, the overall picture was that of an initial inflammatory phase with edema, which progressed into a phase of lung parenchyma loss, and then resolved with scar tissue formation (Fig. 5d, f). Alveolar structure had disappeared and was replaced by granulation tissue (edematous phase; Fig. 5e). The acute phase of inflammation (inflammatory phase) had subsided, and permanent scar tissue was evident in the alveolar septae (scar formation; Fig. 5f).

Scanning electron microscopic findings in the PI-instilled lungs

There was extensive and severe leukocyte infiltration and alveolar destruction in the PI-instilled lung 1 h after the instillation. This infiltration and destruction were less extensive and severe in the PI-instilled lungs at 7 and 14 days after the instillation. Fourteen days after PI instillation, the alveoli were atelectatic or ruptured (Fig. 6g, i). Twenty-one days after the intrabronchial instillation of 0.1% or 5% PI, the shape of the alveoli was abnormal, and many were atelectatic or ruptured (Fig. 6b, c, e, f). No abnormal changes were observed in the SEM images of the PBS-instilled lungs (Fig. 6a, d).

Cytotoxicity of PI in the lungs

The viability of lung tissue following 1-day exposure to 0.01%, 0.1%, 1%, or 5% PI progressively decreased in a significant dose-dependent manner (70.2% ± 13.4%, 47.0% ± 10.4%, 36.2% ± 7.5%, and 30.7% ± 3.8%; $P < 0.05$; Fig. 7-2). When the PBS-treated cultured lung tissues were histologically examined, the microarchitecture of the lung tissue was preserved (Fig. 7-1a). However, the damage to the alveoli and bronchial epithelium of the PI-treated lung tissues showed a dose-dependent pattern of injury (Fig. 7-1b–1d).

Discussion

The cytotoxic and antiseptic mechanisms of action of PI are well known [13]. The antimicrobial mechanism of PI action is attributed to the iodination and oxidation of SH-, OH-, and NH-groups in amino acids and the double bonds

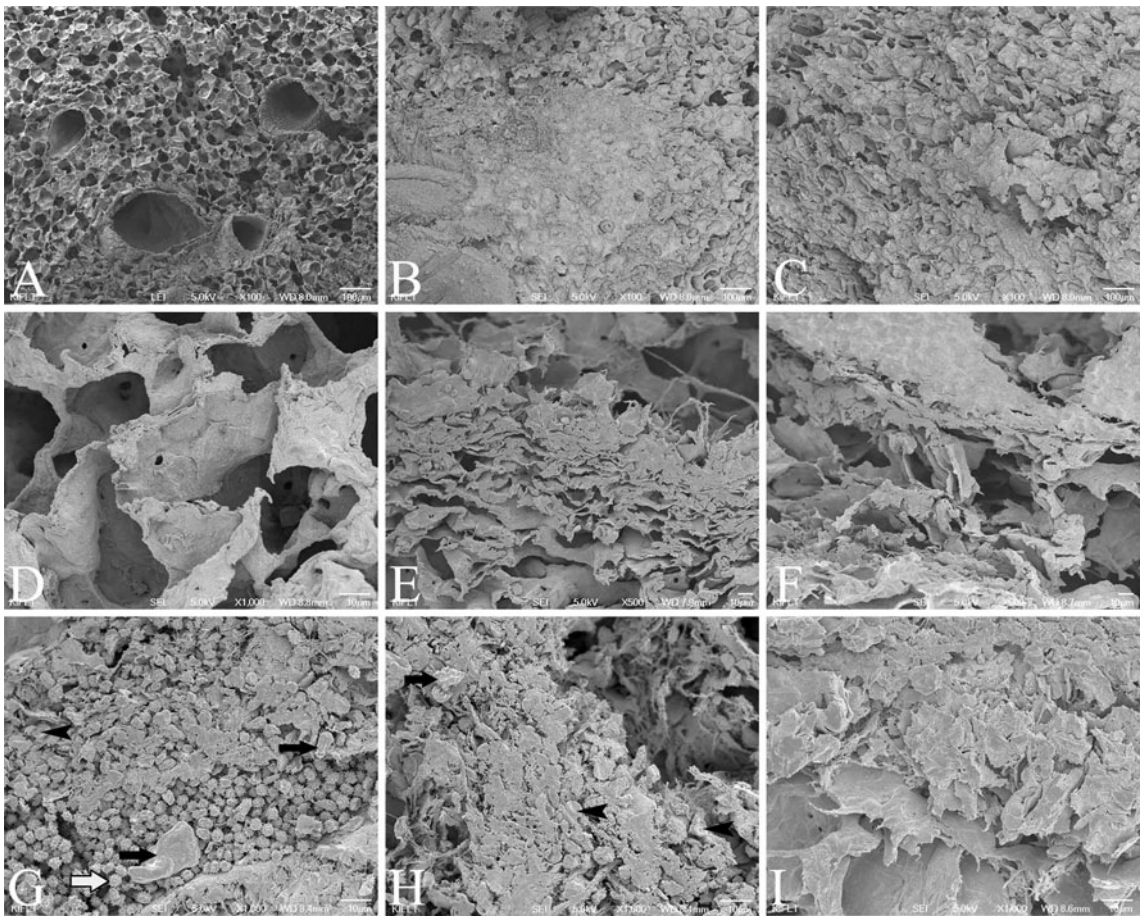
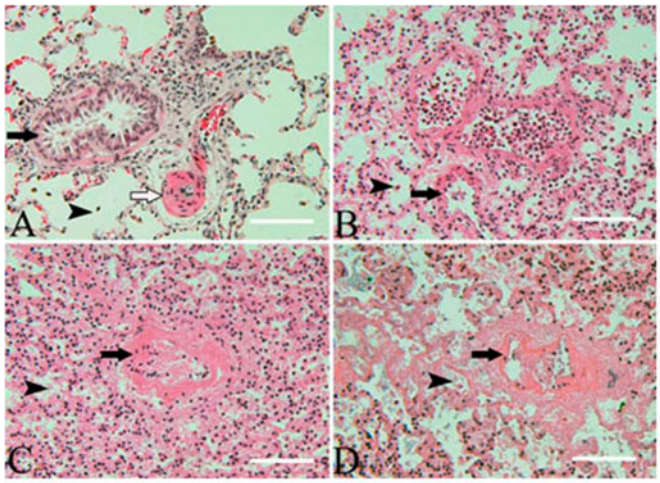
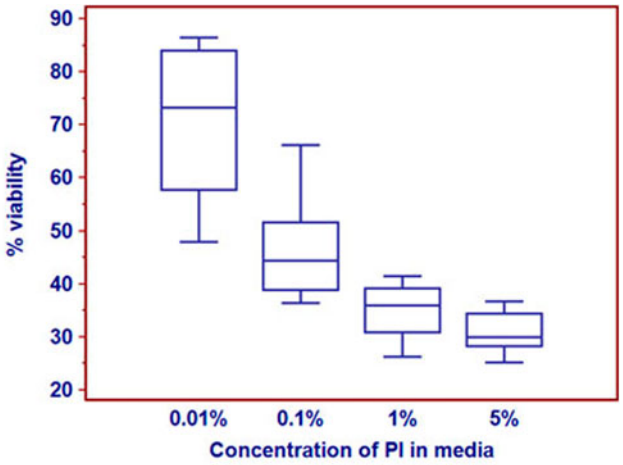


Fig. 6 Scanning electron microscopy of lung tissue 21 days after intrabronchial instillation of 0.2 ml phosphate-buffered saline (PBS) (**a**, **d**), or 0.2 ml 0.1% povidone-iodine (PI) (**b**, **e**) or 5% PI solution (**c**, **f**). Scanning electron micrographs of lung tissue 1 h (**g**), 7 days (**h**), and 14 days (**i**) after the intrabronchial instillation of 0.2 ml 5% PI solution. *Black arrow*, PI-like pigment; *white arrow*, leukocyte; *arrowhead*, red blood cell. **a–c** ×100; **d** ×1,000; **e**, **f** ×500; **g–i** ×1,000. *Bar* **a–c** 100 μm; **d–i** 10 μm

in unsaturated fatty acids in the membranes and cytoplasm of the infective agents [14, 15]. A 10% PI solution generally contains 90% water, 8.5% PI, and 1% available iodine and iodide, and is a potent bactericidal solution that does not irritate the oral mucosa [16]. However, this solution is potentially cytotoxic and cytocidal for mammalian cells because of its nonspecific action, which has been attributed to the oxidization of free iodine in an aqueous solution [17]. Iwasaki et al. [18] studied PI cytotoxicity in cultured Chinese hamster lung V79 cells and reported that PI cytotoxicity occurs in a dose- and treatment time-dependent manner. They also reported that PI induces inhibition of DNA, cellular RNA, and protein synthesis, even at doses and treatment times when PI was not cytotoxic. Although a 5% PI solution is thought to be a safe and effective antiseptic when used on skin and mucous membranes, the results of our study have shown that an intrabronchial instillation of PI can induce lung injury. This injury encompasses hemorrhage, edema, pneumonia, pneumonitis, and fibrosis, and its severity and extent appear to be dose related, and sometimes fatal. Howe [8] reported a case in which a 34-year-old man developed aspiration pneumonia from PI after preparation of the oral cavity for facial surgery. Rales were heard bilaterally in the upper lung approximately 5 min after completing the oral preparation. The patient was mechanically ventilated in the intensive care unit for 30 h after the event. While in the unit, the vital signs of the patient were stable and no fever developed. Radiologic examination of the chest revealed clearing of the upper lobe infiltrates 5 days after the aspiration, and the patient recovered without any complications or the development of further sequelae. In a second case, Numazawa et al. [9] described a case in which pneumonia occurred in a patient after PI aspiration during surgery under general anesthesia. In their report, the surgeon had used 60 ml 0.7% PI solution to clean the oral cavity, and an unknown volume of the PI solution was aspirated because of an insufficient seal by the inflated endotracheal tube cuff. Radiologic examination of the patient's chest revealed signs of pneumonia and atelectasis in the right



1. Microscopic findings in the lungs after 24-hours incubation with PI.



2. MTT [3-[4,5-dimethylthiazol]-2, 5-diphenyl tetrazolium bromide] assay of lung tissue after a 24-hours incubation with PI.

Fig. 7 Cytotoxic effect on lung by povidone-iodine (PI). **1** PI-treated lung tissue shows sloughing of respiratory bronchial epithelium (*b*), necrosis of bronchial wall (*arrow*) (*c*), and confluent necrosis of alveolar and bronchial walls (*d*). *Black arrow*, bronchus; *white arrow*, vessels; *arrowhead*, alveoli. *Bars* 300 μm. **2** Untreated lung tissues were used as viability (100%) controls. The results are expressed as percentage viability relative to control

upper lobe, which progressively improved during the following 1 week. In our study, edematous-like lesions in the lungs were seen in the radiographs of the lungs 1 day after instillation of the PI solution. Although these lesions were not seen when the radiologic examination was repeated 5 days later, macroscopic lesions were found when the lungs were harvested after 21 days. These findings suggest that PI causes pulmonary edema immediately after its instillation, and the edema resolves with time. However, the instillation of PI also elicits an inflammatory reaction in the lungs with leukocyte infiltration and results in atelectasis within 1 h after its instillation. With time, this inflammatory response progressively dissipates, and the resultant lung lesion is scar tissue. Because no lung biopsies were done on the two patients that were described in the case reports of Howe [8] and Numazawa et al. [9], it is not possible to conclude that the lung injury did not persist after PI aspiration in these two patients. In our study, we found that the rats whose lungs were intratracheally instilled with 0.5 ml of 5% PI solution died, whereas those rats whose lungs were intratracheally instilled with 0.5 ml PI solution of less than 1% survived. Although we cannot explain the exact cause of death immediately after the administration of the 5% PI solution, we presume that this difference was the result of differences in the severity of the injury to the alveolar capillary network after PI instillation. Light and scanning electron microscopic examinations of the lung microarchitecture 1 h after the instillation of a 5% PI solution showed severe destruction of the alveolar capillary network and flooding of leukocytes into the alveolar and interstitial spaces. Moreover, no clear distinction in the extent or severity of the pulmonary injury was evident in the lungs that were instilled with 1% PI or 5% PI solution in the microscopic assessment. In the preliminary study, we instilled sequentially decreasing PI concentrations into the lungs and found dose-dependent differences in the severity and extent of the pulmonary lesions. We also found macroscopic lesions in the lungs that were instilled with a PI solution whose strength was greater than 0.1%. However, we did find a difference in the macroscopic findings in the lungs that were instilled with 0.1% PI solution and 5% PI solution, and this difference suggests that the lung injury is dose dependent. We also found a dose-dependent pattern of lung cytotoxicity for PI concentrations in the ex vivo MTT assay. It is difficult to say that the results in ex vivo MTT assays actually reflect the in vivo cytotoxicity of PI in the lung. We performed a pilot study in vivo to obtain quantitative analysis results of cytotoxicity of PI in the lung. However, the rats whose lungs were instilled with more than 0.2 ml 5% PI died immediately after the instillation. Moreover, it is very difficult to obtain lung tissue for even distribution of PI in surviving rats whose lungs were instilled with less than 0.2 ml of various concentrations of PI. To overcome these difficulties, we performed ex vivo MTT assays, which are believed to support in vivo results. Collectively, the results of these assessments and the cytotoxic assay imply that the severity and extent of PI-induced lung injury is dependent on the PI dose. This study has some limitations. The microscopic findings that we have reported may not be

representative of other structural changes in the lung because we only microscopically examined the macroscopic lesions. We also did not do lung function studies in the rats. Hence, we are unable to comment on any physiological changes to lung function after PI instillation. We also did not do bronchoalveolar lavage after PI instillation to establish whether this procedure would minimize the pulmonary injury by lowering the PI concentration. We presume that the instillation of many chemicals or liquids into the lung will also cause atelectasis and other types of lung injury. We did not do a comparative study using other oral cleansing agents to establish whether PI was less or more harmful than any other oral disinfectant. In our study, we used pulmonary atelectasis as the macroscopic marker of lung injury after pulmonary PI instillation in the rats. Owing to the presence of atelectasis and the concomitant occurrence of death after pulmonary PI installation in some rats, a prospective study to assess the severity of PI-induced lung injury cannot be conducted in human subjects. However, we can assume that the PI doses that were used in our experiment reflect the severity and extent of the pulmonary lung injury after PI aspiration in humans.

Conclusions

In summary, various concentrations of a pulmonary instilled PI solution can induce lung injury, including pulmonary fibrosis. Considering that PI will continue to be useful for antiseptic of the oral cavity, users of PI solution should be aware of the aspiration hazard and its potential to cause lung injury.

Conflict of interest The authors report no declarations of conflict of interest.

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