las Pharm Inc. He is also the recipient of research grants from AstraZeneca K.K., Daiichi-Sankyo Co., Takeda Pharmaceutical Co. Ltd., Otsuka Pharmaceutical Co. Ltd., Astellas Pharm Inc., and Eisai Co. Takashi Joh has received service honoraria from AstraZeneca K.K., Daiichi-Sankyo Co., Takeda Pharmaceutical Co. Ltd., Astellas Pharm Inc., MSD K.K., and Mitsubishi Tanabe Pharme Corporation. Hiroshi Nagata, Juntaro Matsuzaki, Toshihiro Nishizawa, Hiroshi Hosoda, Soichiro Miura have no conflicts to declare. Jan Tack has acted as a scientific advisor to Addex, Almirall, AstraZeneca, Danone, GlaxoSmithKline, Ironwood, Menarini, Novartis, sanofi-aventis, Shire, SK Life Sciences, Takeda, Theravance, Tranzyme Pharma, XenoPort and Zeria, and has undertaken speaking engagements for Abbott, Alfa Wasserman, Almirall, AstraZeneca, Dainippon Sumimoto, Janssen, Menarini, Novartis, Nycomed, Takeda and Shire.

AUTHOR CONTRIBUTION

HS and MK designed the research; HS, JM, YF, FS, KK, TN, YN, TH, TK, TA, HY, YT, HN, MM, KK, SM, NU, and TJ contributed to the participant acquisition; HS, JM, MK, and TT conducted the statistical analyses; HH conducted the assay for ghrelin; HS, JM, and JT drafted the manuscript; all authors contributed to the interpretation of data, revised it critically and approved the final version of the manuscript.

REFERENCES

1 Tack J, Talley NJ, Camilleri M, Holtmann G, Hu P, Malagelada JR, Stanghellini V. Functional gastroduodenal disorders. *Gastroenterology* 2006; **130**: 1466–79.

2 Tatsuta M, Iishi H. Effect of treatment with liu-jun-zi-tang (TJ-43) on gastric emptying and gastrointestinal symptoms in dyspeptic patients. *Aliment Pharmacol Ther* 1993; **7**: 459–62.

3 Tominaga K, Iwakiri R, Fujimoto K, Fujiwara Y, Tanaka M, Shimoyama Y, Umegaki E, Higuchi K *et al.* Rikkunshito improves symptoms in PPI-refractory GERD patients: a prospective, randomized, multicenter trial in Japan. *J Gastroenterol* 2012; **47**: 284–92.

4 Suzuki H, Inadomi JM, Hibi T. Japanese herbal medicine in functional gastrointestinal disorders. *Neurogastroenterol Motil* 2009; **21**: 688–96.

5 Yanai M, Mochiki E, Ogawa A, Morita H, Toyomasu Y, Ogata K, Tabe Y, Ando H *et al.* Intragastric administration of rikkunshito stimulates upper gastrointestinal motility and gastric emptying in conscious dogs. *J Gastroenterol* 2013; **48**: 611–9.

6 Furukawa N, Manabe N, Kase Y, Hattori T, Imamura H, Kusunoki H, Haruma K. Intragastric infusion of rikkunshito (kampo) induces proximal stomach relaxation in conscious dogs. *Auton Neurosci* 2013; **179**: 14–22.

7 Tominaga K, Kido T, Ochi M, Sadakane C, Mase A, Okazaki H, Yamagami H, Tanigawa T *et al.* The traditional Japanese medicine rikkunshito promotes gastric emptying via the antagonistic action of the 5-HT(3) receptor pathway in rats. *Evid Based Complement Alternat Med* 2011; **2011**: 248481.

8 Shiratori M, Shoji T, Kanazawa M, Hongo M, Fukudo S. Effect of rikkunshito on gastric sensorimotor function under distention. *Neurogastroenterol Motil* 2011; **23**: 323–9, e155-6.

9 Kusunoki H, Haruma K, Hata J, Ishii M, Kamada T, Yamashita N, Honda K, Inoue K *et al.* Efficacy of Rikkunshito, a traditional Japanese medicine (Kampo), in treating functional dyspepsia. *Intern Med* 2010; **49**: 2195–202.

10 Takeda H, Sadakane C, Hattori T, Katsurada T, Ohkawara T, Nagai K, Asaka M. Rikkunshito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT2 receptor antagonism. *Gastroenterology* 2008; **134**: 2004–13.

11 Takeda H, Muto S, Nakagawa K, Ohnishi S, Sadakane C, Saegusa Y, Nahata M, Hattori T *et al.* Rikkunshito as a ghrelin enhancer. *Methods Enzymol* 2012; **514**: 333–51.

12 Arai M, Matsumura T, Tsuchiya N, Sadakane C, Inami R, Suzuki T, Yoshikawa M, Imazeki F *et al.* Rikkunshito improves the symptoms in patients with functional dyspepsia, accompanied by an increase in the level of plasma ghrelin. *Hepatogastroenterology* 2012; **59**: 62–6.

13 Sadakane C, Muto S, Nakagawa K, Ohnishi S, Saegusa Y, Nahata M, Hattori T, Asaka M *et al.* 10-Gingerol, a component of rikkunshito, improves cisplatin-induced anorexia by inhibiting acylated ghrelin degradation. *Biochem Biophys Res Commun* 2011; **412**: 506–11.

14 Matsumura T, Arai M, Yonemitsu Y, Maruoka D, Tanaka T, Suzuki T, Yoshikawa M, Imazeki F *et al.* The traditional Japanese medicine Rikkunshito increases the plasma level of ghrelin in humans and mice. *J Gastroenterol* 2010; **45**: 300–7.

15 Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656–60.

16 Asakawa A, Inui A, Fujimiya M, Sakamaki R, Shinfuku N, Ueta Y, Meguid MM, Kasuga M. Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut* 2005; **54**: 18–24.

17 Suzuki H, Masaoka T, Sakai G, Ishii H, Hibi T. Improvement of gastrointestinal quality of life scores in cases of *Helicobacter pylori*-positive functional dyspepsia after successful eradication therapy. *J Gastroenterol Hepatol* 2005; **20**: 1652–60.

18 Matsueda K, Hongo M, Tack J, Saito Y, Kato H. A placebo-controlled trial of acotiamide for meal-related symptoms of functional dyspepsia. *Gut* 2012; **61**: 821–8.

19 Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; **40**: 373–83.

20 Veldhuyzen van Zanten SJ, Chiba N, Armstrong D, Barkun A, Thomson A, Smyth S, Escobedo S, Lee J *et al.* A randomized trial comparing omeprazole, ranitidine, cisapride, or placebo in *Helicobacter pylori* negative, primary care patients with dyspepsia: the CADET-HN Study. *Am J Gastroenterol* 2005; **100**: 1477–88.

21 Suzuki H, Masaoka T, Hosoda H, Ota T, Minegishi Y, Nomura S, Kangawa K, Ishii H. *Helicobacter pylori* infection modifies gastric and plasma ghrelin dynamics in Mongolian gerbils. *Gut* 2004; **53**: 187–94.

22 Suzuki H, Masaoka T, Hosoda H, Nomura S, Ohara T, Kangawa K, Ishii H, Hibi T. Plasma ghrelin concentration correlates with the levels of serum pepsinogen I and pepsinogen I/II ratio–a possible novel and non-invasive marker for gastric atrophy. *Hepatogastroenterology* 2004; **51**: 1249–54.

23 Kido T, Nakai Y, Kase Y, Sakakibara I, Nomura M, Takeda S, Aburada M. Effects of rikkunshi-to, a traditional Japanese medicine, on the delay of gastric emptying induced by N(G)-nitro-L-arginine. *J Pharmacol Sci* 2005; **98**: 161–7.
24 Kito Y, Suzuki H. Properties of Rikkunshi-to (TJ-43)-induced relaxation of rat gastric fundus smooth muscles. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G755–63.
25 Tack J, Caenepeel P, Fischler B, Piessevaux H, Janssens J. Symptoms associated with hypersensitivity to gastric distention in functional dyspepsia. *Gastroenterology* 2001; **121**: 526–35.
26 Araki Y, Mukaisho KI, Fujiyama Y, Hattori T, Sugihara H. The herbal medicine rikkunshito exhibits strong and differential adsorption properties for bile salts. *Exp Ther Med* 2012; **3**: 645–9.
27 Gunji S, Ueda S, Yoshida M, Kanai M, Terajima H, Takabayashi A. Effects of rikkunshito, a kampo medicine, on quality of life after proximal gastrectomy. *J Surg Res* 2013; **185**: 575–80.
28 Suzuki H, Moayyedi P. *Helicobacter pylori* infection in functional dyspepsia. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 168–74.
29 Suzuki H, Matsuzaki J, Hibi T. What is the difference between *Helicobacter pylori*-associated dyspepsia and functional dyspepsia? *J Neurogastroenterol Motil* 2011; **17**: 124–30.
30 Suzuki H, Nishizawa T, Hibi T. Can *Helicobacter pylori*-associated dyspepsia be categorized as functional dyspepsia? *J Gastroenterol Hepatol* 2011; **26**(Suppl. 3): 42–5.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of analyzed patients (per-protocol population).

Table S2. Differences in baseline characteristics between participants in hospitals and those in primary care clinics.

Table S3. Difference in efficacy of Rikkunshito between genders (intention-to-treat population).

Table S4. Alterations of plasma ghrelin levels (mean ± SD).

APPENDIX

Members in the rikkunshito study group are Hidekazu Suzuki (Keio University Hospital, Shinjuku, Tokyo), Kunio Kasugai (Aichi Medical University Hospital, Nagakute, Aichi), Toshihiro Nishizawa (Tokyo Medical Centre, Meguro, Tokyo), Yuji Naito (Kyoto Prefectural University of Medicine, Kyoto), Takashi Joh (Nagoya City University, Nagoya, Aichi), Soichiro Miura (National Defense Medical College, Tokorozawa, Saitama), Naomi Uemura (Konodai Hospital, National Center for Global Health and Medicine, Ichikawa, Chiba), Yasushi Fukushima (Tokyo Station Centre Clinic, Chuo-ku, Tokyo), Fumio Suzaki (Yokohama Minami Kyosai Hospital, Yokohama, Kanagawa), Toshihiko Hayakawa (Hayakawa Clinic, Tokai, Aichi), Takashi Ando (Social Insurance Kyoto Hospital, Kyoto), Hideo Yoshida (Eiju Hospital, Taito-ku, Tokyo), Yoshifumi Tokura (Tokura Clinic, Kameoka, Kyoto), Hiroshi Nagata (Keiyu Hospital, Yokohama, Kanagawa), Masao Kobayakawa (National Center for Global Health and Medicine Center Hospital, Shinjuku, Tokyo), Mikiji Mori (Ogikubo Clinic, Suginami, Tokyo), Kimihiko Kato (Meito Hospital, Nagoya, Aichi), Yoshikazu Tsuzuki (Tsuzuki Clinic, Tachikawa, Tokyo), Akihiro Iwata (Iwata Naika Clinic, Nagoya, Aichi), Kunio Kobayashi (Kobayashi Naika Clinic, Nagoya, Aichi), Hiroyuki Imaeda (Department of General Medicine, Saitama Medical University, Iruma, Saitama), Yasuharu Yamaguchi (Yamaguchi Clinic, Nishi-Tokyo, Tokyo), Eisuke Iwasaki (Division of Internal Medicine, Saiseikai Central Hospital, Minato-ku, Tokyo), Hirokazu Komatsu (Division of Gastroenterology, Yokohama Municipal Hospital, Yokohama), Hiroshi Serizawa (Kitasato University Kitasato Institute Hospital, Minato-ku, Tokyo), Yuichi Oyamada (Matsushita Memorial Hospital, Osaka), Yuzo Yagou (Yagou Clinic, Itabashi, Tokyo), Takeshi Yoshida (Saitama Social Insurance Hospital, Saitama), Jiro Nishida (Tokyo Dental College Ichikawa General Hospital, Ichikawa, Chiba), Sayuri Yamamoto (Yuri Clinic, Chita, Aichi), and Kazuhiko Tokita (Yosanoumi Hospital, Kyoto).

1521-0103/350/1/79–88$25.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

http://dx.doi.org/10.1124/jpet.114.213009
J Pharmacol Exp Ther 350:79–88, July 2014

Ameliorative Effect of Mepenzolate Bromide against Pulmonary Fibrosis[S]

Shota Kurotsu, Ken-ichiro Tanaka, Tomomi Niino, Teita Asano, Toshifumi Sugizaki, Arata Azuma, Hidekazu Suzuki, and Tohru Mizushima

Department of Analytical Chemistry, Faculty of Pharmacy, Keio University, Tokyo, Japan (S.K., K.-i.T., T.N., T.A., T.S., T.M.); Department of Internal Medicine, Division of Respiratory, Infection and Oncology, Nippon Medical School, Tokyo, Japan (A.A.); and Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan (H.S.)

Received January 10, 2014; accepted April 23, 2014

ABSTRACT

Idiopathic pulmonary fibrosis is thought to involve lung injury caused by reactive oxygen species (ROS), which in turn is followed by abnormal fibrosis. A transforming growth factor (TGF)-β1–induced increase in myofibroblast number plays an important role in this abnormal fibrosis. We recently found that mepenzolate bromide (mepenzolate), which has been used clinically to treat gastrointestinal disorders, has ROS-reducing properties. In the present study, we examined the effect of mepenzolate on bleomycin-induced pulmonary fibrosis and lung dysfunction in mice. The severity of pulmonary fibrosis was assessed by histopathologic evaluation and determination of hydroxyproline levels. Lung mechanics (elastance) and respiratory function [forced vital capacity (FVC)] were assessed using a computer-controlled ventilator. Respiratory function was also evaluated by monitoring percutaneous arterial oxygen saturation (SpO_2). Intratracheal administration of mepenzolate prior to bleomycin treatment reduced the extent of pulmonary fibrosis and changes in lung mechanics and led to a significant recovery of both FVC and SpO_2 compared with control. Furthermore, mepenzolate produced a therapeutic effect even when it was administered after the development of fibrosis. Administration of mepenzolate also prevented bleomycin-induced pulmonary cell death and inflammatory responses and increased myofibroblast number. Mepenzolate also decreased NADPH oxidase activity and active TGF-β1 level or increased glutathione S-transferase (GST) activity in the presence of bleomycin treatment. These results show that the intratracheal administration of mepenzolate reduced bleomycin-induced pulmonary fibrosis and lung dysfunction in mice. These effects may be due to this drug's inhibitory effect on NADPH oxidase and TGF-β1 activities and its stimulatory effect on GST.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive and devastating chronic lung condition with poor prognosis; the reported mean length of survival from the time of diagnosis is 2.8–4.2 years. IPF progresses insidiously and slowly, and acute exacerbations of the condition are highly lethal (Kim et al., 2006; Raghu et al., 2011). Current agents for the treatment of IPF, such as steroids and immunosuppressors, have not been found to improve prognosis (Luppi et al., 2004; Walter et al., 2006; Raghu et al., 2011). Pirfenidone, a novel antifibrotic drug, was reported in some (but not all) clinical studies to slow the rate of forced vital capacity (FVC) decrease in patients with IPF. Although this drug is licensed in Japan and Europe as a treatment of IPF (Azuma et al., 2005; Maher, 2010; Taniguchi et al., 2010), the Food and Drug Administration declined to approve its use because of inconclusive evidence of its clinical efficacy and because of severe side effects, such as photosensitivity in dermatitis and nausea and anorexia (Maher, 2010; Noble et al., 2011). Thus, the development of new types of drugs to treat IPF is warranted.

Although the etiology of IPF is not yet fully understood, recent studies have suggested that it is triggered by reactive oxygen species (ROS)–induced lung injury and is promoted by abnormal wound repair and remodeling, resulting in abnormal fibrosis (collagen deposition) (Maher et al., 2007; du Bois, 2010). The body contains a number of endogenous antioxidant proteins such as superoxide dismutase (SOD) and glutathione S-transferase (GST) that offer protection against

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Health, Labour, and Welfare of Japan, as well as the Center of Innovation Program from Japan Science and Technology Agency and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.
dx.doi.org/10.1124/jpet.114.213009.
[S] This article has supplemental material available at jpet.aspetjournals.org.

ABBREVIATIONS: BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disease; CPFE, combined pulmonary fibrosis and emphysema; CS, cigarette smoke; DAPI, 4,6-diamidino-2-phenylindole; DMBA, 4-(dimethylamino)-benzaldehyde; DTPA, diethylenetriamine-*N,N,N′,N″,N‴*-pentaacetic acid; FVC, forced vital capacity; GST, glutathione S-transferase; IPF, idiopathic pulmonary fibrosis; PBS, phosphate-buffered saline; ROS, reactive oxygen species; α-SMA, smooth muscle actin; SOD, superoxide dismutase; SpO_2, percutaneous arterial oxygen saturation; TCA, trichloroacetic acid; TGF, transforming growth factor; TUNEL, UTP terminal deoxynucleotidyl transferase–mediated digoxigenindeoxyuridine nick-end labeling.

ROS-induced tissue damage. In addition to ROS, transforming growth factor (TGF)-β1 also seems to play an important role in the pathogenesis of IPF (Sheppard, 2006; Kinnula, 2008). Lung myofibroblasts, consisting of a cell type that is intermediate between fibroblasts and smooth muscle cells, produce considerable amounts of extracellular matrix components, such as collagen, which may give rise to abnormal fibrosis (Hinz et al., 2007). Myofibroblasts are produced by both the activation of fibroblasts and the transformation of epithelial cells through the process of epithelial-mesenchymal transition (Hinz et al., 2007). TGF-β1 appears to increase the number of lung myofibroblasts by activating fibroblasts and inducing epithelial-mesenchymal transition of epithelial cells (Willis and Borok, 2007; Strieter and Mehrad, 2009). It has also been reported that ROS can activate TGF-β1 (Barcellos-Hoff and Dix, 1996; Bellocq et al., 1999).

The coexistence of emphysema and fibrosis in the same patient is known as combined pulmonary fibrosis and emphysema (CPFE) syndrome. The prognosis for CPFE syndrome is especially poor, and an optimal treatment protocol for patients with this syndrome has not been established (Jankowich and Rounds, 2012). This is because pulmonary fibrosis and emphysema are characterized by distinct clinical, radiologic, pathologic, and functional characteristics. For example, pulmonary fibrosis and emphysema increase and decrease lung elastance, respectively (Papiris et al., 2013). On the other hand, since ROS-induced pulmonary damage also plays an important role in the pathogenesis of pulmonary emphysema (Nadeem et al., 2005; Mak, 2008), drugs that could decrease the pulmonary level of ROS may also be effective for treating both pulmonary fibrosis and emphysema. Supporting this notion, we recently reported that inhalation of lecithinized SOD ameliorates both bleomycin-induced pulmonary fibrosis and elastase- or cigarette smoke (CS)–induced pulmonary emphysema by decreasing the pulmonary level of ROS (Tanaka et al., 2010a, 2011, 2012a,b).

We also reported that mepenzolate bromide (mepenzolate), an orally administered muscarinic receptor antagonist used to suppress the gastrointestinal hypermotility associated with irritable bowel syndrome (Long and Keasling, 1954; Buckley et al., 1957; Chen, 1959), could prevent elastase- or CS-induced pulmonary emphysema in mice by decreasing the pulmonary level of ROS (Tanaka et al., 2013). As for the mechanism governing the ROS-reducing activity of mepenzolate, we found that this activity is independent of the muscarinic receptor, because other muscarinic receptor antagonists such as ipratropium bromide (ipratropium) and tiotropium bromide (tiotropium) could not exert ameliorative effects against elastase-induced pulmonary disorders; scopolamine and pirenzepine also have no discernible effects against elastase-induced pulmonary disorders, even though, as for mepenzolate, these drugs are orally administered drugs used to treat gastrointestinal disorders, and their clinical doses are similar to that of mepenzolate; the dose of mepenzolate required to affect elastase-induced pulmonary disorders was much higher than that required to cause the bronchodilation activity (Tanaka et al., 2013). On the other hand, we suggested that the ROS-reducing activity of mepenzolate is mediated by both the inhibition of NADPH oxidase activity and the stimulation of GST activity (Tanaka et al., 2013). Based on these findings, we proposed that mepenzolate could serve as a candidate drug for the treatment of patients with pulmonary emphysema.

In the present study, we examined the effect of mepenzolate on bleomycin-induced pulmonary fibrosis in mice. The results obtained show that the intratracheal administration of mepenzolate suppresses bleomycin-induced pulmonary fibrosis and lung dysfunction in a manner that is probably mediated by this drug's inhibitory effect on NADPH oxidase and TGF-β1 activities and by its stimulatory effect on GST.

Materials and Methods

Chemicals and Animals. Chloramine-T, 4-(dimethylamino)-benzaldehyde (DMBA), potassium dichromate, phosphotungstic acid, mepenzolate, phosphomolybdic acid, orange G, ipratropium, and acid fuchsin were obtained from Sigma-Aldrich (St. Louis, MO). Apocynin was from Santa Cruz Biotechnology (Santa Cruz, CA). Tiotropium was from ChemReagents (Sugarland, TX). Bleomycin was purchased from Nippon Kayaku (Tokyo, Japan), Novo-Heparin (5000 units) was from Mochida Pharmaceutical Co. (Tokyo, Japan), chloral hydrate was from Nacalai Tesque (Kyoto, Japan), and Diff-Quik was from the Sysmex Corporation (Kobe, Japan). The enzyme-linked immunosorbent assay (ELISA) kit for quantifying TGF-β1 was from R&D Systems (Minneapolis, MN), and the assay kit for GST was from PromoKine (Heidelberg, Germany). Antibody against α-smooth muscle actin (α-SMA) was purchased from Abcam (Cambridge, Cambridgeshire, UK), and Alexa Fluor 594 goat anti-rabbit immunoglobulin G was from Invitrogen (Carlsbad, CA). L-Hydroxyproline, sodium acetate, trichloroacetic acid (TCA), azophloxin, aniline blue, and formalin neutral buffer solution were obtained from Wako Pure Chemicals (Tokyo, Japan). Mounting medium for immunohistochemical analysis (VECTASHIELD) was purchased from Vector Laboratories (Burlingame, CA), and Mayer's hematoxylin, 1% eosin alcohol solution, mounting medium for histologic examination (malinol), and Weigert's iron hematoxylin were from Muto Pure Chemicals (Tokyo, Japan). Diethylenetriamine-*N,N,N′,N″,N‴*-pentaacetic acid (DTPA) and 4,6-diamidino-2-phenylindole (DAPI) were purchased from Dojindo (Kumamoto, Japan). Xylidine Ponceau was from Waldeck GmbH & Co. KG, Division Chroma (Muenster, Germany). Isoflurane was from Pfizer (New York, NY). ICR mice (5–6-week-old male) were purchased from Charles River (Yokohama, Japan). The experiments and procedures described here were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health and were approved by the Animal Care Committee of Keio University.

Treatment of Mice with Bleomycin and Mepenzolate. Mice maintained under anesthesia with isoflurane were intratracheally administered bleomycin (5 or 3 mg/kg, once only) in phosphate-buffered saline (PBS) or mepenzolate (various doses) in PBS via a micropipette (P200; Gilson, Inc., Middleton, WI). The first administration of mepenzolate was performed 1 hour before the bleomycin administration (with the exception of experiments shown in Fig. 2).

Preparation of Bronchoalveolar Lavage Fluid, Cell Count, and Measurement of Enzyme Activities and TGF-β1. Bronchoalveolar lavage fluid (BALF) was collected by cannulating the trachea and lavaging the lung with 1 ml of sterile PBS containing 50 units/ml heparin (two times). Approximately 1.8 ml of BALF was routinely recovered from each animal. The total cell number was counted using a hemocytometer. Cells were stained with Diff-Quik reagents after centrifugation with Cytospin 4 (Thermo Fisher Scientific, Inc., Waltham, MA), and the ratios of macrophages and neutrophils to total cells were determined.

NADPH oxidase activity was measured by using lucigenin as a substrate (chemiluminescence) (Griendling et al., 1994). Samples were incubated with 0.1 mM NADPH in 50 mM phosphate buffer containing 1 mM EGTA, 150 mM sucrose, and 0.5 mM lucigenin, and lucigenin chemiluminescence was recorded for 15 minutes in a microplate reader (MicroLumat Plus LB96V; Berthold Technologies, Bad Wildbad, Germany). The GST activity and the amount of active

TGF-β1 were measured by using each assay kit according to the manufacturer's protocol.

Histologic and Immunohistochemical Analyses. Lung tissue samples were fixed in 10% formalin neutral buffer solution for 24 hours, after which they were embedded in paraffin before being cut into 4-μm-thick sections. For histologic examination, sections were stained first with Mayer's hematoxylin and then with 1% eosin alcohol solution (H&E staining). Samples were mounted with malinol and inspected with the aid of an Olympus BX51 microscope.

For staining of collagen (Masson's trichrome staining), sections were treated sequentially with solution A (5% w/v potassium dichromate and 5% w/v TCA), Weigert's iron hematoxylin, solution B (1.25% w/v phosphotungstic acid and 1.25% w/v phosphomolybdic acid), 0.75% w/v Orange G solution, solution C (0.12% w/v xylidine Ponceau, 0.04% w/v acid fuchsin, and 0.02% w/v azophloxin), 2.5% w/v phosphotungstic acid, and finally with aniline blue solution. Samples were mounted with malinol and inspected with the aid of an Olympus BX51 microscope.

For immunohistochemical analysis, sections were blocked with 2.5% goat serum for 10 minutes and then incubated for 12 hours with antibodies against α-SMA (1:100 dilution) in the presence of 2.5% bovine serum albumin, followed by incubation with Alexa Fluor 594 goat anti-rabbit immunoglobulin G (1:500 dilution) and DAPI (5 μg/ml) for 2 hours. Samples were mounted with VECTASHIELD and inspected with the aid of a fluorescence microscope (Olympus BX51).

For TdT-mediated biotinylated UTP terminal deoxynucleotidyl transferase–mediated digoxigenindeoxyuridine nick-end labeling (TUNEL) assay, sections were incubated first with proteinase K for 15 minutes at 37°C and then with TdT and biotin 14-ATP for 1 hour at 37°C, and finally for 2 hours with Alexa Fluor 488 conjugated with streptavidin (1:500 dilution). Samples were mounted with VECTASHIELD and inspected using fluorescence microscopy (Olympus IX73; Olympus Corporation, Tokyo, Japan).

Measurement of Percutaneous Arterial Oxygen Saturation. Measurement of percutaneous arterial oxygen saturation (SpO_2) was performed with the MouseOx system (Starr Life Sciences Corp., Allison Park, PA), as described previously (Tanaka et al., 2012a). The MouseOx sensor attached to the thigh of a mouse under anesthesia with chloral hydrate (500 mg/kg). All data were analyzed using MouseOx software (Starr Life Sciences Corp.).

Measurement of Lung Mechanics and FVC. Measurement of lung mechanics was performed with a computer-controlled small animal ventilator (FlexiVent; SCIREQ, Montreal, QC, Canada), as described previously (Tanaka et al., 2012a). Mice were anesthetized with chloral hydrate (500 mg/kg), a tracheotomy was performed, and an 8-mm-long section of metallic tube (outer and inner diameters of 1.27 and 0.84 mm, respectively) was inserted into the trachea. Mice were mechanically ventilated at a rate of 150 breaths/min, using a tidal volume of 8.7 ml/kg and a positive end-expiratory pressure of 2–3 cm H_2O.

Total respiratory system elastance and tissue elastance were measured by the snap shot and forced oscillation techniques, respectively. All data were analyzed using FlexiVent software (version 5.3; SCIREQ).

Determination of FVC was performed with the same computer-controlled small-animal ventilator connected to a negative pressure reservoir (SCIREQ), as described previously (Tanaka et al., 2012a). Mice were anesthetized and then tracheotomized and ventilated as described earlier. The lungs were inflated to 30 cm H_2O over 1 second and held at this pressure. After 0.2 second, the pinch valve (connected to the ventilator) was closed, and after 0.3 second, the shutter valve (connected to the negative pressure reservoir) was opened, exposing the lung to the negative pressure, which was held for 1.5 seconds to ensure complete expiration. FVC was determined using FlexiVent software (version 5.3).

Hydroxyproline Determination. Hydroxyproline content was determined as described previously (Woessner, 1961). In brief, the lung was removed and homogenized in 0.5 ml of 5% TCA. After centrifugation, pellets were hydrolyzed in 0.5 ml of 10 N HCl for 16 hours at 110°C. Each sample was incubated for 20 minutes at room temperature after the addition of 0.5 ml of 1.4% w/v chloramine-T solution and then incubated at 65°C for 10 minutes after the addition of 0.5 ml of Ehrlich's reagent (1 M DMBA, 70% v/v isopropanol, and 30% v/v perchloric acid). Absorbance was measured at 550 nm, and the amount of hydroxyproline was determined.

Statistical Analysis. All values are expressed as the mean ± S.E.M. One-way analysis of variance followed by the Tukey test or Student's t test for unpaired results were used to evaluate differences between three or more groups or between two groups, respectively. Differences were considered to be significant at P values <0.05.

Results

Effects of Mepenzolate on Bleomycin-Induced Pulmonary Fibrosis. Pulmonary fibrosis was induced by administering mice a single (on day 0) intratracheal dose of bleomycin. To begin with, we examined the preventive effect of mepenzolate on pulmonary fibrosis; mepenzolate was administered intratracheally once daily for 11 days (from day 0 to day 10), and pulmonary fibrosis was assessed on day 21 by histopathological analysis and measurement of pulmonary hydroxyproline levels (an indicator of collagen levels). Histopathological analysis of pulmonary tissue following H&E staining revealed that severe pulmonary damage was induced by the bleomycin and that this damage was suppressed by the intratracheal administration of mepenzolate (Fig. 1A). Masson's trichrome staining of collagen revealed that bleomycin-induced increase in collagen deposition was clearly suppressed by the mepenzolate treatment (Fig. 1, A and B). We also found that the bleomycin-induced elevation of pulmonary hydroxyproline was significantly suppressed by mepenzolate in a dose-dependent manner (Fig. 1C). The administration of mepenzolate did not affect pulmonary hydroxyproline levels in mice that had not been subjected to bleomycin treatment (Fig. 1C).

Changes in lung mechanics associated with pulmonary fibrosis are characterized by an increase in elastance (Tanaka et al., 2010b). Total respiratory system elastance (elastance of the total lung, including the bronchi, bronchioles, and alveoli) and tissue elastance (elastance of the alveoli) increased following bleomycin treatment. These effects were suppressed by the intratracheal administration of mepenzolate, again in a dose-dependent manner (Fig. 1D).

We next examined the effect of mepenzolate on respiratory function. As shown in Fig. 1E, FVC was clearly decreased in bleomycin-treated mice, and this decrease could be significantly reversed with concomitant mepenzolate treatment. We also evaluated respiratory function by monitoring SpO_2, which showed a decrease in bleomycin-treated mice and subsequent return toward control values following treatment with mepenzolate (Fig. 1F). In summary, the results in Fig. 1 show that the intratracheal administration of mepenzolate prevents bleomycin-induced pulmonary damage and fibrosis, and reduces alterations in lung mechanics and respiratory dysfunction.

We next tested the efficacy of mepenzolate when the treatment protocol was initiated after the development of fibrosis (i.e., 10 days after the administration of bleomycin), with pulmonary fibrosis, lung mechanics, and respiratory function parameters assessed on day 21. We first suggested that pulmonary fibrosis and alterations of lung mechanics

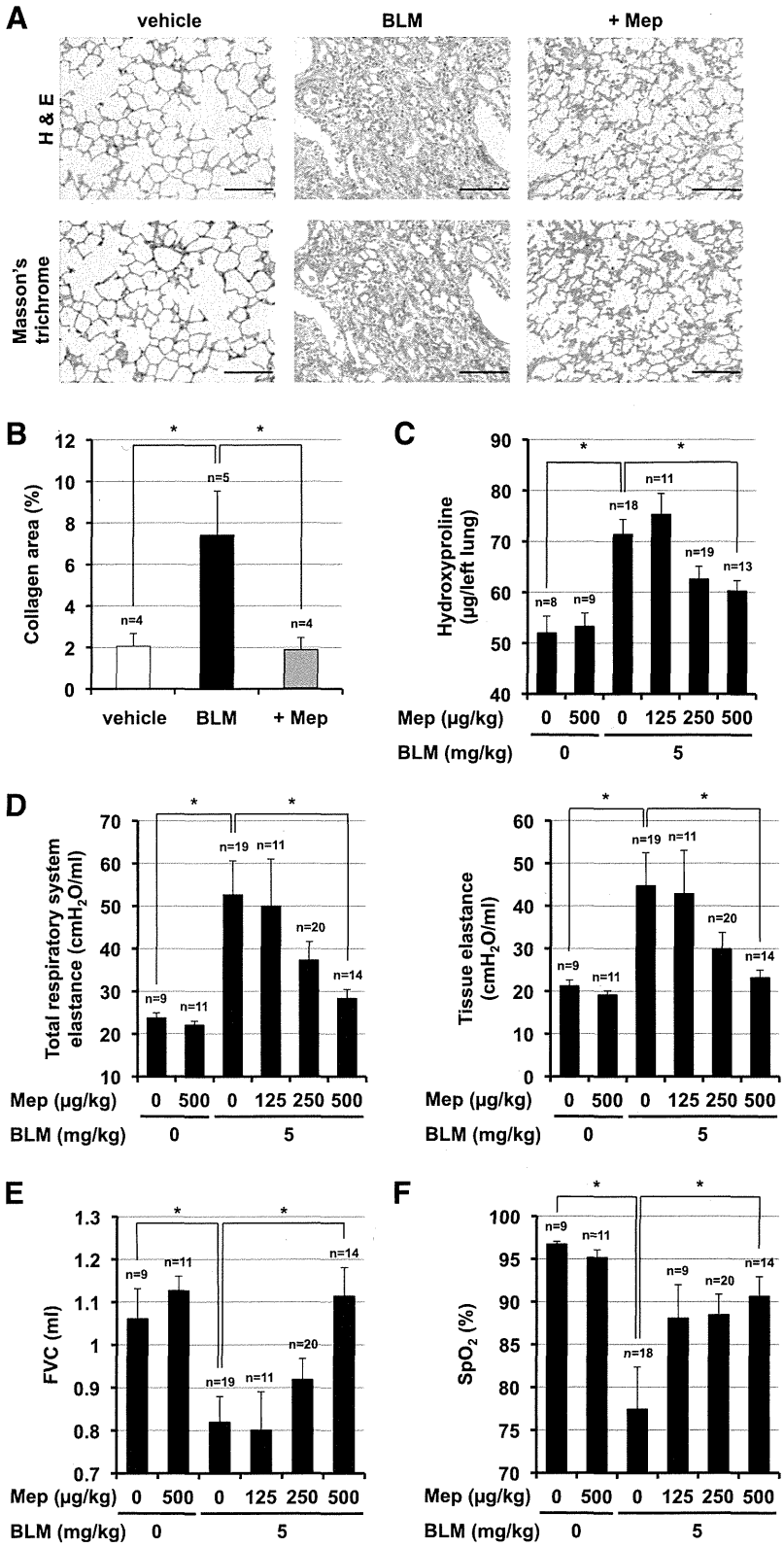

Fig. 1. Effect of mepenzolate on bleomycin-induced pulmonary fibrosis and lung dysfunction. Mice were treated with bleomycin (BLM, 5 mg/kg) or vehicle once only on day 0. Mepenzolate (Mep) at 500 μg/kg (A and B) or the indicated dose (C–F) was administered intratracheally once daily for 11 days (from day 0 to day 10). Sections of pulmonary tissue were prepared on day 21 and subjected to histopathological examination [H&E staining (top images) and Masson's trichrome staining (bottom images); scale bar, 100 μm] (A), and the percentage of area stained with Masson's trichrome was determined using ImageJ software (B). Pulmonary hydroxyproline levels were determined on day 21 (C). Measurement of total respiratory system elastance (D), tissue elastance (D), FVC (E), and SpO_2 (F) was carried out on day 21 as described under *Materials and Methods*. Values represent the mean ± S.E.M. $^*P < 0.05$.

and respiratory function occurred on day 10 under these conditions, and the extent of these alterations on day 10 was less apparent than on day 21, although the differences were not statistically significant (Supplemental Fig. 1). We subsequently found that treatment with mepenzolate (from day 10 to day 19) decreased the extent of pulmonary damage, pulmonary fibrosis, and lung elastance changes on day 21 (Fig. 2, A–C). However, it should be noted that, although mepenzolate showed a tendency to restore FVC in the presence of bleomycin treatment, the recovery was not

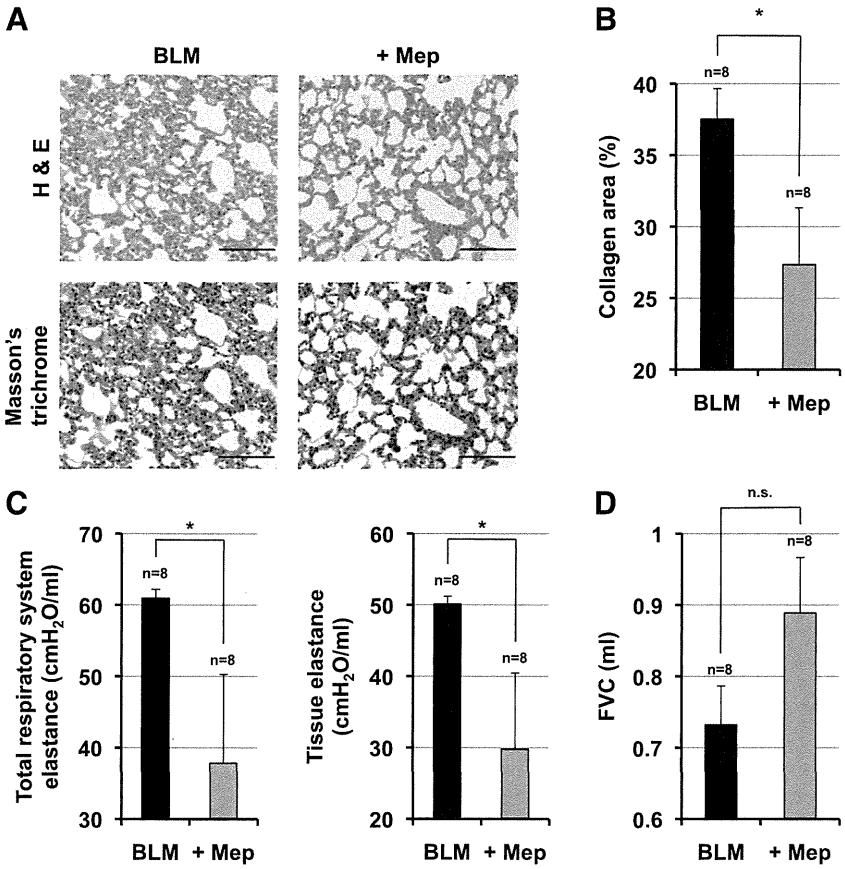

Fig. 2. Effect of mepenzolate on predeveloped pulmonary fibrosis. Mice were treated with bleomycin (BLM, 3 mg/kg) or vehicle on day 0 only. Mepenzolate (Mep, 500 μg/kg) was administered intratracheally once daily for 10 days (from day 10 to day 19). Sections of pulmonary tissue were prepared on day 21 and subjected to histopathological examination [H&E staining (top images) and Masson's trichrome staining (bottom images); scale bar, 100 μm] (A), and the percentage of area stained with Masson's trichrome was determined (B). Measurement of total respiratory system elastance (C), tissue elastance (C), and FVC (D) was carried out on day 21. Values represent the mean ±S.E.M. *$P < 0.05$; n.s., not significant.

statistically significant (Fig. 2D). We showed that the bronchodilatory effect of mepenzolate does not directly affect the monitoring of lung mechanics and respiratory function, based on observations in Supplemental Fig. 2 that intratracheal administration of mepenzolate from day 10 to day 19 did not affect the lung mechanics and respiratory function monitored on day 21 in control mice (without treatment with bleomycin; same washout period as that in Fig. 2, C and D). Results in Fig. 2 indicate that mepenzolate could be an effective agent for the treatment of pre-existing pulmonary fibrosis.

Effects of Mepenzolate on Bleomycin-Induced Pulmonary Cell Death, Inflammatory Responses, and Increase in Myofibroblast Number. As described earlier, pulmonary fibrosis involves various phenomena, such as pulmonary cell death, inflammation, and an increase in myofibroblast number. To this end, we next examined the manner in which mepenzolate affects these bleomycin-induced physiologic changes.

The level of pulmonary cell death was monitored by TUNEL assay. Although treatment with bleomycin increased the number of TUNEL-positive cells, this increase was suppressed by the simultaneous administration of mepenzolate (Fig. 3).

Next, we monitored bleomycin-induced pulmonary inflammatory responses by determining the number of leukocytes in BALF. As shown in Fig. 4A, the total number of leukocytes increased following bleomycin treatment, an effect which was partially suppressed by the concomitant treatment of animals with mepenzolate. Similar results were observed in relation to the numbers of macrophages and neutrophils (Fig. 4A).

We also used immunohistochemical analysis with antibodies against α-SMA, a marker for myofibroblasts (Hinz et al., 2007), to examine the effect of mepenzolate on the pulmonary level of myofibroblasts. As shown in Fig. 4, B and C, bleomycin administration increased the number of α-SMA–positive cells, whereas simultaneous treatment with mepenzolate decreased this level, thus indicating that this drug suppresses the bleomycin-induced increase in lung myofibroblast number.

As described earlier, we reported that, in an elastase-induced pulmonary emphysema model of mice, anti-inflammatory and ROS-reducing activities of mepenzolate are independent of its muscarinic receptor–mediated bronchodilatory activity (Tanaka et al., 2013). To test the contribution of the bronchodilatory activity of mepenzolate to its ameliorative effect against bleomycin-induced pulmonary fibrosis, we examined the effect of other muscarinic antagonists (bronchodilators), such as ipratropium and tiotropium, on bleomycin-induced pulmonary fibrosis and alteration of lung mechanics and respiratory functions. Since we previously reported that bronchodilatory activity was indistinguishable between mepenzolate and ipratropium (Tanaka et al., 2013), here we used the ipratropium dose (500 μg/kg) equivalent to that of mepenzolate that is required to suppress bleomycin-induced pulmonary fibrosis (Fig. 1) and the tiotropium dose (56 μg/kg), considering the clinical doses of ipratropium and tiotropium. As shown in Fig. 5, intratracheal administration of each of these muscarinic antagonists could not suppress bleomycin-induced pulmonary fibrosis and alteration of lung mechanics and

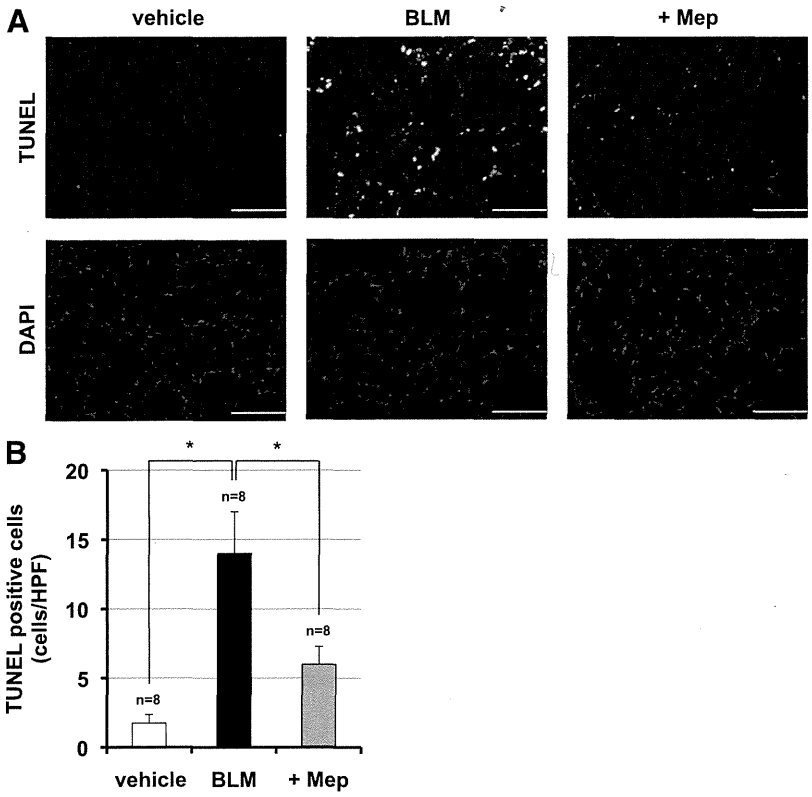

Fig. 3. Effect of mepenzolate on bleomycin-induced pulmonary cell death. Mepenzolate (Mep, 500 μg/kg) was administered intratracheally once only. Mice were treated with bleomycin (BLM, 5 mg/kg) or vehicle 1 hour after the mepenzolate administration. Sections of pulmonary tissue were prepared 24 hours after the BLM administration and subjected to TUNEL assay and DAPI staining (A). TUNEL-positive cells were counted (B). Values represent the mean ± S.E.M. $^*P < 0.05$. HPF, high-power field.

respiratory functions, suggesting that the ameliorative effect of mepenzolate against bleomycin-induced pulmonary fibrosis is independent of its muscarinic receptor–mediated bronchodilatory activity.

Effects of Mepenzolate on NADPH Oxidase, GST, and TGF-β1. As described earlier, we recently reported that mepenzolate decreases the pulmonary level of ROS by suppressing the NADPH oxidase activation and by stimulating GST activity in elastase- or CS-administered mice (Tanaka et al., 2013). With the results of those studies in mind, we next examined the effect of mepenzolate on NADPH oxidase and GST activities in bleomycin-administered mice. As shown in Fig. 6A, treatment with bleomycin increased pulmonary NADPH activity in a manner that could be partially suppressed by the simultaneous administration of mepenzolate. Administration of mepenzolate increased pulmonary GST activity in the presence of bleomycin treatment (Fig. 6B). Although treatment with bleomycin showed a tendency to decrease GST activity, the decrease was not statistically significant (Fig. 6B).

To test the contribution of the inhibitory effect of mepenzolate on NADPH oxidase activity to its ameliorative effect against bleomycin-induced pulmonary damage, we examined the effect of apocynin (an inhibitor of NADPH oxidase) and/or mepenzolate on bleomycin-induced pulmonary cell death, inflammatory responses, and increase in myofibroblast number. As shown in Fig. 7, intratracheal administration of apocynin suppressed bleomycin-induced pulmonary cell death, inflammatory responses, and increase in myofibroblast number; however, this administration of apocynin did not affect these indexes in the presence of treatment with mepenzolate, suggesting that the ameliorative effect of mepenzolate against bleomycin-induced pulmonary damage is mediated by its inhibitory effect on NADPH oxidase.

Finally, we monitored the pulmonary level of active TGF-β1, which is also an important endogenous factor implicated in promoting pulmonary fibrosis (see earlier discussion). Treatment with bleomycin increased the pulmonary level of active TGF-β1 as described previously (Takemasa et al., 2012), and this increase was suppressed by the simultaneous administration of mepenzolate (Fig. 6C). The results in Figs. 6 and 7 thus suggest that the ameliorative effect of mepenzolate on bleomycin-induced pulmonary fibrosis is mediated by its inhibitory effect on NADPH oxidase and TGF-β1 activities, and by its stimulatory effect on GST activity.

Discussion

As IPF is a disease that affects lung mechanics and respiratory function, it is important to examine the effect that a candidate drug has not only on pulmonary fibrosis but also on these other parameters. We have shown here that the concomitant administration of mepenzolate can reduce bleomycin-induced pulmonary fibrosis, increased lung elastance, and respiratory dysfunction seen with bleomycin treatment. The extent of amelioration by mepenzolate was similar to that afforded by pirfenidone (a drug used clinically to treat patients with IPF) or lecithinized human Cu/Zn-superoxide dismutase (a drug being developed to treat IPF), which were investigated in the same animal model under similar conditions (Tanaka et al., 2010a, 2012a). Furthermore, in terms of clinical relevance, it is important to examine not only the preventive value of candidate compounds but also their therapeutic efficacy; to this extent, we found that mepenzolate is effective in combating pre-existing pulmonary fibrosis. Taken together, these results suggest that mepenzolate could be beneficial for the treatment of patients with IPF.

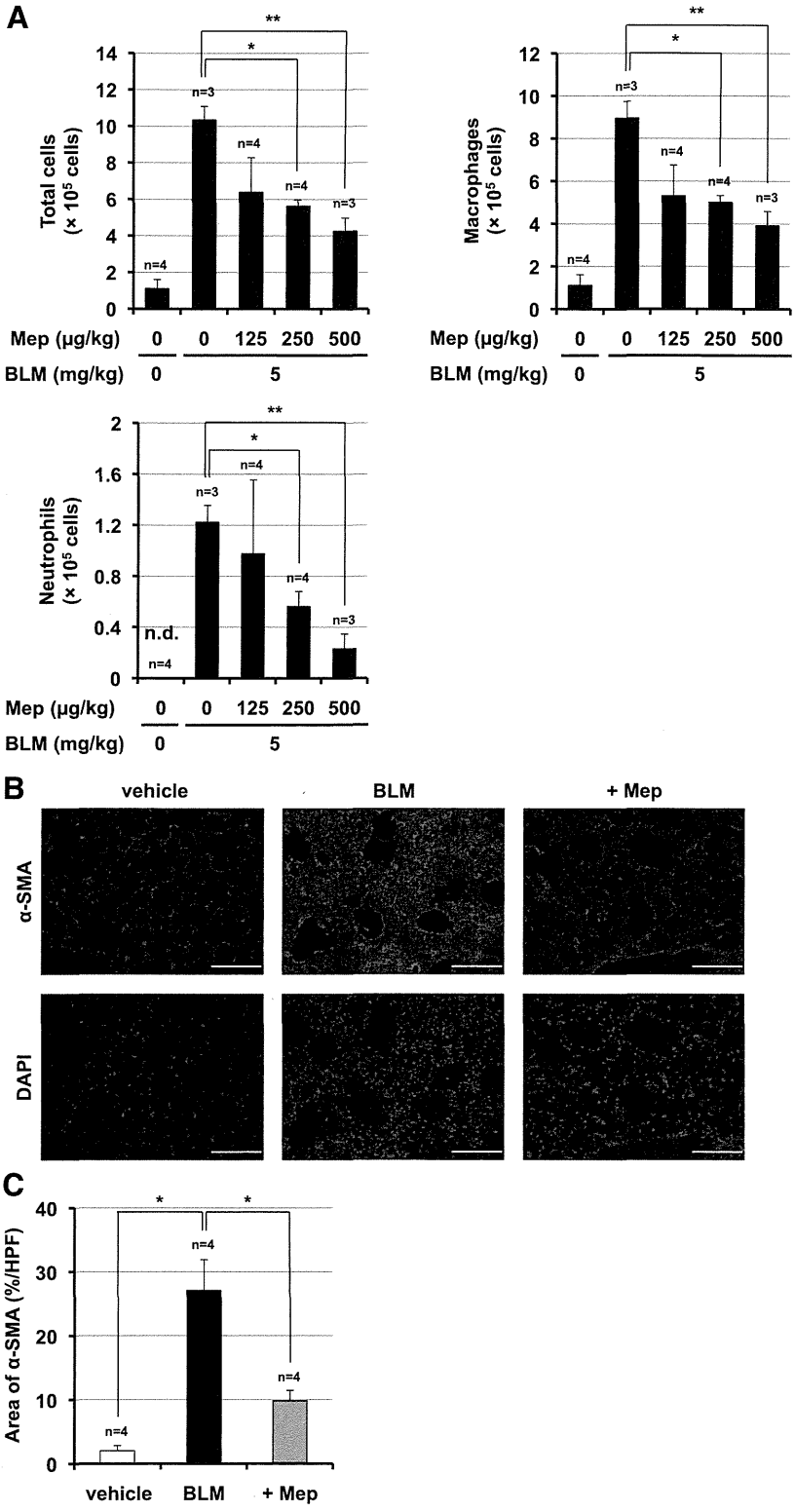

Fig. 4. Effect of mepenzolate on bleomycin-induced pulmonary inflammatory responses and increase in myofibroblast number. Mice were treated with bleomycin (BLM, 5 mg/kg) or vehicle once only on day 0. Mepenzolate (Mep) at the indicated dose (A) or 500 μg/kg (B and C) was administered intratracheally once daily for 10 days (from day 0 to day 9). Total cell number and individual numbers of macrophages and neutrophils in BALF were determined on day 10 as described under *Materials and Methods* (A). Sections of pulmonary tissue were prepared on day 10 and subjected to immunohistochemical analysis with an antibody against α-SMA (scale bar, 100 μm) (B). The percentage of area stained with the antibody was determined using ImageJ software (C). Values represent the mean ± S.E.M. $^{**}P < 0.01$; $^{*}P < 0.05$. HPF, high-power field; n.d., not determined.

To understand the mechanism governing the ameliorative effect of mepenzolate on pulmonary fibrosis, we also examined its effects on bleomycin-induced pulmonary cell death and inflammatory responses, as well as increase in myofibroblast number, and found that mepenzolate could suppress all of these phenomena. To understand the mechanism at the molecular level, we focused on NADPH oxidase, GST, and TGF-β1, given that ROS play an important role in causing the pulmonary cell damage associated with IPF, and that TGF-β1 can increase the number of lung myofibroblasts (Kinnula and Myllarniemi, 2008; Strieter and Mehrad, 2009). Furthermore, we recently reported that mepenzolate not only inhibited NADPH oxidase activity but also stimulated GST activity in elastase- or CS-administered mice (Tanaka et al., 2013). Here,

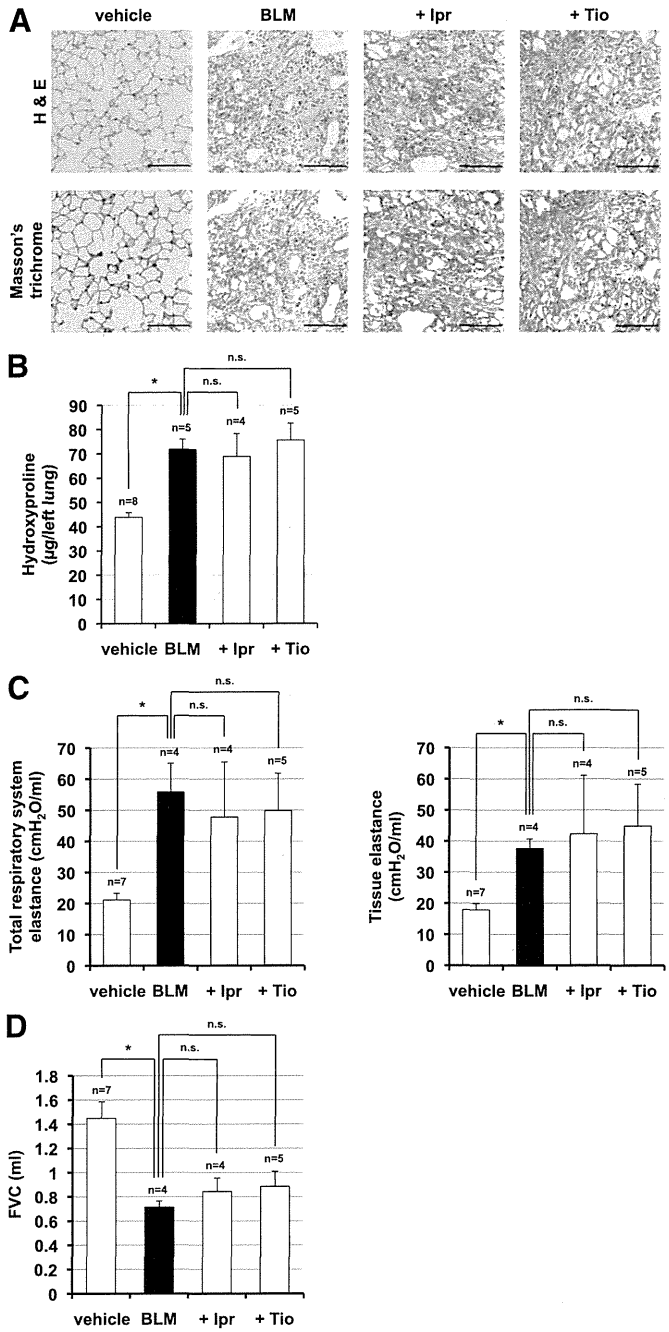

Fig. 5. Effect of other muscarinic antagonists on bleomycin-induced pulmonary fibrosis and lung dysfunction. Mice were treated with bleomycin (BLM, 5 mg/kg) or vehicle once only on day 0. Ipratropium (Ipr, 500 μg/kg) or tiotropium (Tio, 56 μg/kg) was administered intratracheally once daily for 11 days (from day 0 to day 10). Sections of pulmonary tissue were prepared on day 21 and subjected to histopathological examination [H&E staining (upper images) and Masson's trichrome staining (lower images); scale bar, 100 μm] (A). Pulmonary hydroxyproline levels were determined on day 21 (B). Measurement of total respiratory system elastance (C), tissue elastance (C), and FVC (D) was carried out on day 21. Values represent the mean ± S.E.M. $^*P < 0.05$; n.s., not significant.

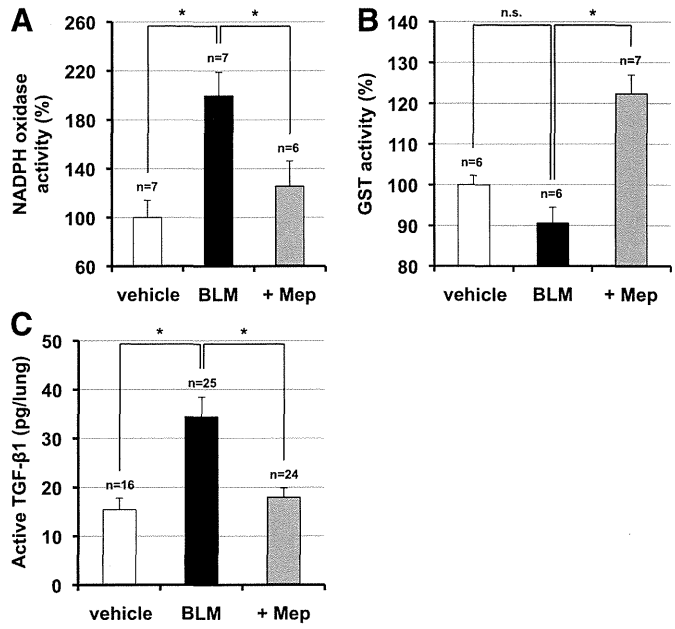

Fig. 6. Effect of mepenzolate on pulmonary activities of NADPH oxidase, GST, and TGF-β1. Mice were treated with bleomycin (BLM, 5 mg/kg) or vehicle once only on day 0. Mepenzolate (Mep, 500 μg/kg) was administered intratracheally once daily for 3 days (from day 0 to day 2) (A), once only on day 0 (B), or once daily for 10 days (from day 0 to day 9) (C). Cells in BALF were prepared on day 3, and NADPH oxidase activity was determined (A). On day 1 (B) or day 10 (C), lung homogenates were prepared and pulmonary levels of GST activity (B) or the active form of TGF-β1 (C) were determined. Values represent the mean ± S.E.M. $^*P < 0.05$; n.s., not significant.

we found that mepenzolate suppressed both the activation of NADPH oxidase and the increase in the active form of TGF-β1 brought about by bleomycin treatment. Furthermore, administration of mepenzolate restored pulmonary GST activity in the presence of bleomycin treatment. The result suggests that these effects are involved in the ameliorative activity of mepenzolate on bleomycin-induced pulmonary fibrosis. Since it was reported that ROS induce the activation of TGF-β1 (Barcellos-Hoff and Dix, 1996; Bellocq et al., 1999), the inhibitory effect of mepenzolate on the pulmonary level of active TGF-β1 can likely be explained by its ROS-reducing activity. On the other hand, although some previous reports suggested that tiotropium shows therapeutic effects on lipopolysaccharide-induced pulmonary inflammatory responses and remodeling in vivo and suppresses acetylcholine-induced proliferation of fibroblasts and myofibroblasts in vitro (Pieper et al., 2007; Pera et al., 2011), here we show that tiotropium is inert for bleomycin-induced pulmonary fibrosis.

As described under *Introduction*, pulmonary fibrosis and emphysema are characterized by distinct clinical, radiologic, pathologic, and functional characteristics. For example, imaging and pathologic examinations highlight the fact that pulmonary fibrosis and emphysema are manifested in different ways in terms of the pulmonary regions involved and the parenchymal modifications that take place (Jankowich and Rounds, 2012). Furthermore, it was believed that pulmonary fibrosis or emphysema involves excess or insufficient wound repair, respectively (Chilosi et al., 2013). For these reasons, the clinical treatment of CPFE syndrome is rendered very difficult. Because ROS are involved in the pathogenesis of both pulmonary fibrosis and emphysema, ROS-decreasing drugs may be effective for treating both conditions. To this end, we found that, as for the animal model of pulmonary emphysema, mepenzolate is effective in treating the animal model of pulmonary fibrosis.

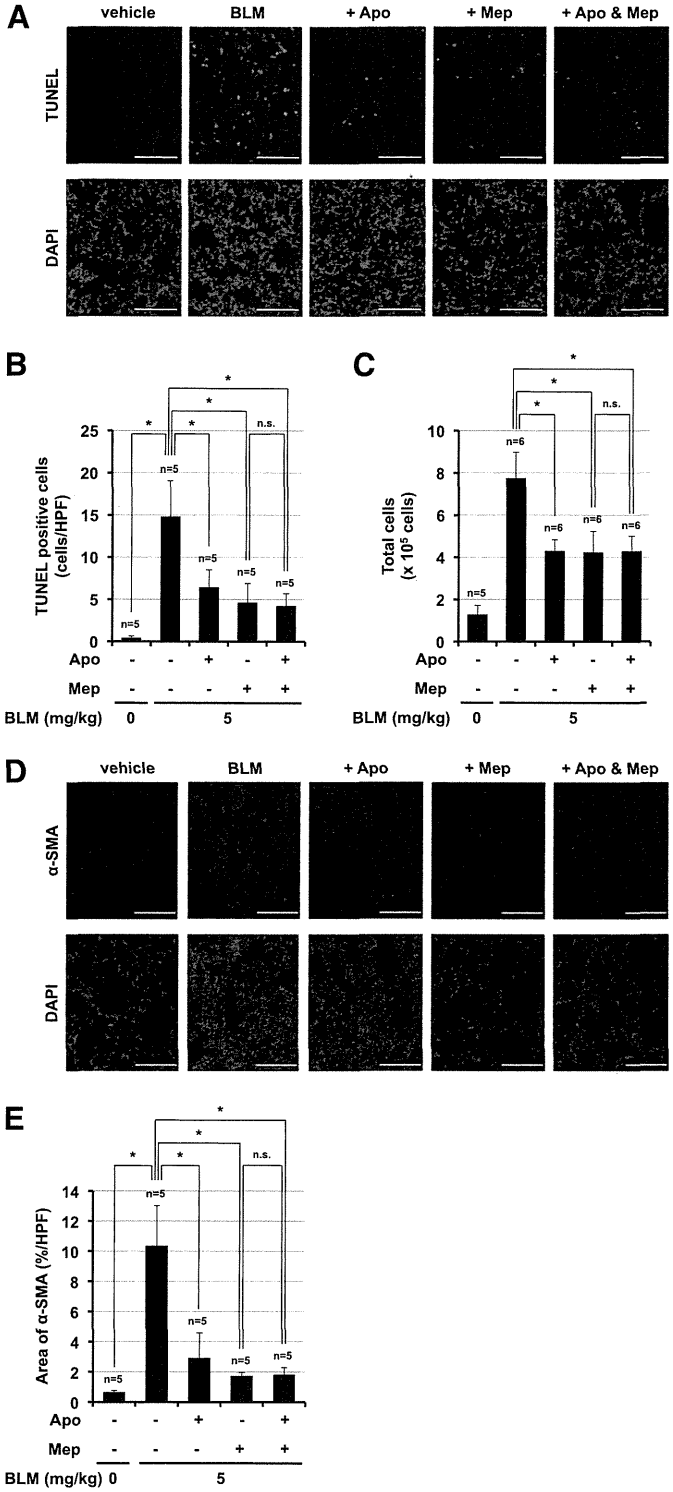

Fig. 7. Effect of mepenzolate and/or apocynin on bleomycin-induced pulmonary cell death, inflammatory responses, and increase in myofibroblast number. Mice were treated with bleomycin (BLM, 5 mg/kg) or vehicle once only on day 0 (A–E). Mepenzolate (Mep, 500 μg/kg) and/or apocynin (Apo, 1 mg/kg) was intratracheally administered once only 1 hour before the BLM administration (A and B) or once daily for 11 days (from day 0 to day 10) (C–E). Sections of pulmonary tissue were prepared 24 hours after the BLM administration and subjected to TUNEL assay and DAPI staining (A). TUNEL-positive cells were counted (B). Total cell number in BALF was determined on day 10 (C). Sections of pulmonary tissue were prepared on day 10 and subjected to immunohistochemical analysis with an antibody against α-SMA (scale bar, 100 μm) (D). The percentage of area stained with the antibody was determined using ImageJ software (E). Values represent the mean ± S.E.M. $^*P < 0.05$; n.s., not significant. HPF, high-power field.

Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation and abnormal inflammatory responses, for which a combination of anti-inflammatory drugs (such as steroids) and bronchodilators forms the standard treatment regimen (Rabe et al., 2007). On the other hand, mepenzolate exhibits not only anti-inflammatory activity but also bronchodilatory activity due to its muscarinic receptor–antagonizing action (Tanaka et al., 2013). Therefore, it is reasonable to postulate that this drug may be beneficial for treating COPD without the concomitant use of other medications. A feasible approach might be to initially develop mepenzolate for the treatment of COPD, followed by the clinical testing of its effects on CPFE syndrome.

The number of drugs reaching the marketplace each year is decreasing, mainly as a consequence of unexpected adverse effects of potential drugs being revealed at advanced clinical trial stages. For this reason, we proposed a new strategy for drug discovery and development (drug repositioning) (Mizushima, 2011). In this strategy, compounds with therapeutically beneficial activity are screened from a library of approved medicines with a view to developing them for new indications. We previously applied this strategy to the development of drugs to treat patients with COPD by testing potential drugs on animal models of COPD (elastase- or CS-induced pulmonary emphysema), with mepenzolate identified as a potential candidate (Tanaka et al., 2013), and in this study, we found that this drug is effective for bleomycin-induced pulmonary fibrosis in mice, which is a useful model for IPF (Moore et al., 2013). Thus, we proposed that mepenzolate may be a good candidate drug for IPF, because its safety has already been confirmed clinically.

Authorship Contributions

Participated in research design: Kurotsu, Tanaka, Mizushima.
Conducted experiments: Kurotsu, Tanaka, Niino, Asano, Sugizaki.
Performed data analysis: Kurotsu, Tanaka.
Wrote or contributed to the writing of the manuscript: Kurotsu, Tanaka, Azuma, Suzuki, Mizushima.

References

Azuma A, Nukiwa T, Tsuboi E, Suga M, Abe S, Nakata K, Taguchi Y, Nagai S, Itoh H, and Ohi M, et al. (2005) Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* **171**: 1040–1047.
Barcellos-Hoff MH and Dix TA (1996) Redox-mediated activation of latent transforming growth factor-beta 1. *Mol Endocrinol* **10**:1077–1083.
Bellocq A, Azoulay E, Marullo S, Flahault A, Fouqueray B, Philippe C, Cadranel J, and Baud L (1999) Reactive oxygen and nitrogen intermediates increase transforming growth factor-beta1 release from human epithelial alveolar cells through two different mechanisms. *Am J Respir Cell Mol Biol* **21**:128–136.
Buckley JP, DeFEO JJ, and Reif EC (1957) The comparative antispasmodic activity of N-methyl-3-piperidyl diphenylglycolate methobromide (JB-340) and atropine sulfate. *J Am Pharm Assoc Am Pharm Assoc (Baltim)* **46**:592–594.
Chen JY (1959) Antispasmodic activity of JB-340 (N-methyl-3-piperidyl-diphenylglycolate methobromide) with special reference to its relative selective action on the sphincter of Oddi, colon and urinary bladder of the dog. *Arch Int Pharmacodyn Ther* **121**:78–84.
Chilosi M, Carloni A, Rossi A, and Poletti V (2013) Premature lung aging and cellular senescence in the pathogenesis of idiopathic pulmonary fibrosis and COPD/emphysema. *Transl Res* **162**:156–173.
du Bois RM (2010) Strategies for treating idiopathic pulmonary fibrosis. *Nat Rev Drug Discov* **9**:129–140.
Griendling KK, Minieri CA, Ollerenshaw JD, and Alexander RW (1994) Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* **74**:1141–1148.
Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, and Gabbiani G (2007) The myofibroblast: one function, multiple origins. *Am J Pathol* **170**:1807–1816.
Jankowich MD and Rounds SI (2012) Combined pulmonary fibrosis and emphysema syndrome: a review. *Chest* **141**:222–231.
Kim DS, Collard HR, and King TE, Jr (2006) Classification and natural history of the idiopathic interstitial pneumonias. *Proc Am Thorac Soc* **3**:285–292.
Kinnula VL (2008) Redox imbalance and lung fibrosis. *Antioxid Redox Signal* **10**: 249–252.

Kinnula VL and Myllärniemi M (2008) Oxidant-antioxidant imbalance as a potential contributor to the progression of human pulmonary fibrosis. *Antioxid Redox Signal* **10**:727–738.
Long JP and Keasling HH (1954) The comparative anticholinergic activity of a series of derivatives of 3-hydroxy piperidine. *J Am Pharm Assoc Am Pharm Assoc (Baltim)* **43**:616–619.
Luppi F, Cerri S, Beghè B, Fabbri LM, and Richeldi L (2004) Corticosteroid and immunomodulatory agents in idiopathic pulmonary fibrosis. *Respir Med* **98**: 1035–1044.
Maher TM (2010) Pirfenidone in idiopathic pulmonary fibrosis. *Drugs Today (Barc)* **46**:473–482.
Maher TM, Wells AU, and Laurent GJ (2007) Idiopathic pulmonary fibrosis: multiple causes and multiple mechanisms? *Eur Respir J* **30**:835–839.
Mak JC (2008) Pathogenesis of COPD. Part II. Oxidative-antioxidative imbalance. *Int J Tuberc Lung Dis* **12**:368–374.
Mizushima T (2011) Drug discovery and development focusing on existing medicines: drug re-profiling strategy. *J Biochem* **149**:499–505.
Moore B, Lawson WE, Oury TD, Sisson TH, Raghavendran K, and Hogaboam CM (2013) Animal models of fibrotic lung disease. *Am J Respir Cell Mol Biol* **49**: 167–179.
Nadeem A, Raj HG, and Chhabra SK (2005) Increased oxidative stress and altered levels of antioxidants in chronic obstructive pulmonary disease. *Inflammation* **29**: 23–32.
Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, Kardatzke D, King TE, Jr, Lancaster L, Sahn SA, and Szwarcberg J, et al. CAPACITY Study Group (2011) Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. *Lancet* **377**:1760–1769.
Papiris SA, Triantafillidou C, Manali ED, Kolilekas L, Baou K, Kagouridis K, and Bouros D (2013) Combined pulmonary fibrosis and emphysema. *Expert Rev Respir Med* **7**:19–31, quiz 32.
Pera T, Zuidhof A, Valadas J, Smit M, Schoemaker RG, Gosens R, Maarsingh H, Zaagsma J, and Meurs H (2011) Tiotropium inhibits pulmonary inflammation and remodelling in a guinea pig model of COPD. *Eur Respir J* **38**:789–796.
Pieper MP, Chaudhary NI, and Park JE (2007) Acetylcholine-induced proliferation of fibroblasts and myofibroblasts in vitro is inhibited by tiotropium bromide. *Life Sci* **80**:2270–2273.
Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, and van Weel C, et al.; Global Initiative for Chronic Obstructive Lung Disease (2007) Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* **176**:532–555.
Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, and Lasky JA, et al.; ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis (2011) An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* **183**:788–824.
Sheppard D (2006) Transforming growth factor beta: a central modulator of pulmonary and airway inflammation and fibrosis. *Proc Am Thorac Soc* **3**:413–417.
Strieter RM and Mehrad B (2009) New mechanisms of pulmonary fibrosis. *Chest* **136**: 1364–1370.
Takemasa A, Ishii Y, and Fukuda T (2012) A neutrophil elastase inhibitor prevents bleomycin-induced pulmonary fibrosis in mice. *Eur Respir J* **40**:1475–1482.
Tanaka K, Azuma A, Miyazaki Y, Sato K, and Mizushima T (2012a) Effects of lecithinized superoxide dismutase and/or pirfenidone against bleomycin-induced pulmonary fibrosis. *Chest* **142**:1011–1019.
Tanaka K, Ishihara T, Azuma A, Kudoh S, Ebina M, Nukiwa T, Sugiyama Y, Tasaka Y, Namba T, and Ishihara T, et al. (2010a) Therapeutic effect of lecithinized superoxide dismutase on bleomycin-induced pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* **298**:L348–L360.
Tanaka K, Ishihara T, Sugizaki T, Kobayashi D, Yamashita Y, Tahara K, Yamakawa N, Iijima K, Mogushi K, and Tanaka H, et al. (2013) Mepenzolate bromide displays beneficial effects in a mouse model of chronic obstructive pulmonary disease. *Nat Commun* **4**:2686.
Tanaka K, Sato K, Aoshiba K, Azuma A, and Mizushima T (2012b) Superiority of PC-SOD to other anti-COPD drugs for elastase-induced emphysema and alteration in lung mechanics and respiratory function in mice. *Am J Physiol Lung Cell Mol Physiol* **302**:L1250–L1261.
Tanaka K, Tanaka Y, Miyazaki Y, Namba T, Sato K, Aoshiba K, Azuma A, and Mizushima T (2011) Therapeutic effect of lecithinized superoxide dismutase on pulmonary emphysema. *J Pharmacol Exp Ther* **338**:810–818.
Tanaka K, Tanaka Y, Namba T, Azuma A, and Mizushima T (2010b) Heat shock protein 70 protects against bleomycin-induced pulmonary fibrosis in mice. *Biochem Pharmacol* **80**:920–931.
Taniguchi H, Ebina M, Kondoh Y, Ogura T, Azuma A, Suga M, Taguchi Y, Takahashi H, Nakata K, and Sato A, et al.; Pirfenidone Clinical Study Group in Japan (2010) Pirfenidone in idiopathic pulmonary fibrosis. *Eur Respir J* **35**:821–829.
Walter N, Collard HR, and King TE, Jr (2006) Current perspectives on the treatment of idiopathic pulmonary fibrosis. *Proc Am Thorac Soc* **3**:330–338.
Willis BC and Borok Z (2007) TGF-beta-induced EMT: mechanisms and implications for fibrotic lung disease. *Am J Physiol Lung Cell Mol Physiol* **293**:L525–L534.
Woessner JF, Jr (1961) The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch Biochem Biophys* **93**:440–447.

Address correspondence to: Dr. Tohru Mizushima, Department of Analytical Chemistry, Faculty of Pharmacy, Keio University, 1-5-30, Shibakoen, Minato-ku, Tokyo 105-8512, Japan. E-mail: mizushima-th@pha.keio.ac.jp

Neurogastroenterology & Motility

Neurogastroenterol Motil (2014) **26**, 764–771 doi: 10.1111/nmo.12319

Greater loss of productivity among Japanese workers with gastro-esophageal reflux disease (GERD) symptoms that persist *vs* resolve on medical therapy

H. SUZUKI,* J. MATSUZAKI,* T. MASAOKA* & J. M. INADOMI†

*Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan
†Department of Internal Medicine, University of Washington, Seattle, WA, USA

Key Messages

- The association between GERD and work productivity has not been well investigated in Japan.
- The impact of GERD on work and daily productivity was evaluated using a Web-based WPAI-GERD questionnaires.
- Participants with persistent GERD symptoms reported a greater loss of work productivities than those whose symptoms were alleviated with medications.

Abstract

Background *Gastro-esophageal reflux disease (GERD) impairs quality of life; however, the association between GERD and work productivity has not been well investigated in Japan. This study was designed to compare the impact of GERD on productivity between Japanese workers with GERD symptoms that persisted vs resolved on medical therapy.* ***Methods*** *A cross-sectional Web-based survey was conducted in workers. The impact of GERD on work and daily productivity was evaluated using a Web-reported Work Productivity and Activity Impairment Questionnaire for patients with GERD and a GERD symptom severity Questionnaire. Demographic information, clinical history, and satisfaction with GERD medication were also ascertained.* ***Key Results*** *A total of 20 000 subjects were invited to the survey. After the exclusion of patients with a history of gastrointestinal (GI) malignancy, peptic ulcer, upper GI surgery, and unemployment, 650 participants were included in the analysis. Participants with persistent GERD symptoms reported a significantly greater losses of work productivity (11.4 ± 13.4 h/week), absenteeism (0.7 ± 3.1 h/week), presenteeism (10.7 ± 12.6 h/week), costs (20 100 ± 26 800 JPY/week), and lower daily productivity (71.3% [95% confidence interval, 69.0–73.7]) than those whose symptoms were alleviated with medications. The level of dissatisfaction with GERD medications among participants with persistent GERD symptoms was significantly correlated with loss of work and daily productivity ($p < 0.001$).* ***Conclusions & Inferences*** *GERD places a significant burden on work and daily productivity despite medical therapy. Ineffective GERD therapy is associated with greater productivity loss.*

Keywords *gastro-esophageal reflux disease, quality of life, treatment satisfaction, work productivity.*

Address for Correspondence
Hidekazu Suzuki, MD, PhD, FACG, Associate Professor, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8382, Japan.
Tel: +81-3-5363-3914; fax: +81-3-5363-3967;
e-mail: hsuzuki@a6.keio.jp
Received: 12 December 2013
Accepted for publication: 21 January 2014

INTRODUCTION

Gastro-esophageal reflux disease (GERD) is a chronic disorder with symptoms that include chronic heartburn and acid regurgitation and often affects patients during periods of activity, leading to a significant impairment of quality of life (QOL).[1] According to the REQUEST study, GERD was associated with a significant decrease in

QOL as determined using the Japanese version of the eight-item Short-Form Health Survey and the reflux esophagitis (RE)-specific health-related QOL questionnaire.[2] The OMAREE study also investigated the impact of RE on QOL in Japanese patients[3] and showed that 75.2% had more than three upper gastrointestinal (GI) symptoms and 31.5% had more than six upper GI symptoms; both groups showed a reduced QOL compared with healthy participants. A prospective cohort study (ProGERD) conducted in Germany using the Reflux Disease Diagnostic Questionnaire also demonstrated a reduced QOL among patients with GERD.[4] In Western countries, GERD symptoms often cause increased absenteeism (reduced total number of working hours) and presenteeism (reduced number of productive work hours), resulting in significant economic losses caused by reduced work productivity and costs incurred by treatments and clinical visits. Self-administered questionnaires are commonly used to determine work-related productivity in patients with GERD. In the United States and Europe, loss of work productivity has been assessed using a Work Productivity and Activity Impairment (WPAI) questionnaire. A Swedish study validated the use of the WPAI for GERD and demonstrated a high level of convergence between GERD symptoms and impaired QOL.[5] Furthermore, several studies revealed that reductions in work productivity were associated with GERD symptom severity.[5–10] Specifically, patients who had GERD symptoms >2 days/week had significantly reduced productivity compared with patients who had symptoms <2 days/week.[8] According to the subanalysis of the RANGE (retrospective analysis of GERD) study conducted in 134 primary care institutes across six European countries, including Germany, Greece, Norway, Spain, Sweden, and the UK,[11] the average absenteeism due to GERD was highest in Germany (3.2 h/week) and lowest in the UK (0.4 h/week), with an average of up to 6.7 additional hours/week lost owing to presenteeism in Norway.

In addition, average economic losses were substantial in all the countries (from €55/week per employed patient in the UK to €273 in Sweden), with reductions in productivity of up to 26% across these European countries. However, no studies have investigated whether work productivity is associated with GERD symptoms or treatment satisfaction in Japanese workers. According to our earlier survey, patients with GERD symptoms frequently take over-the-counter (OTC) drugs for heartburn and/or regurgitation in addition to prescribed medication.[12] This previous web survey indicated that 45% of Japanese patients with GERD who took prescription medication also used OTC drugs more than once a week.[12] Patients with symptomatic GERD might be a burden in terms of productivity losses in work and daily life.

Thus, the aims of this study were to investigate productivity and economic losses in working and in daily life activities using a Japanese version of the GERD symptom severity Questionnaire (GerdQ) and WPAI-GERD Questionnaire, in Japanese workers with GERD symptoms and to compare GERD patients whose symptoms were persistent *vs* alleviated with medications.

MATERIALS AND METHODS

Study design

This cross-sectional study assessed the association between work productivity and GerdQ scores, comorbidities, current medications, or treatment satisfaction. This study was approved by the institutional ethics review board of Keio University School of Medicine (No. 20120201). All the participants in the study provided informed consent. Ethical guidelines followed the Declaration of Helsinki and Ethical Guidelines for Epidemiological Research (issued by the Ministry of Health, Labour and Welfare of Japan [MHLW]). In this study, GERD was defined as patients with GerdQ scores ≥8 or patients who were using drugs to treat reflux symptoms. Reflux symptoms mean heartburn or acid regurgitation.

Participants

Participants (n = 20 000), who have or had symptoms as heartburn or regurgitation, registered in a research program owned and managed by a survey company (MACROMILL Co., Tokyo, Japan) were sent an e-mail invitation to participate in this survey. Men and women aged 20–69 years with symptoms of GERD were included. The survey included questions about participant height, weight, occupation, current and past medical history, past surgical history, GerdQ questionnaire,[12,13] WPAI-GERD questionnaire, current medication (prescription and OTC), and treatment satisfaction (if applicable). Different from Western countries, PPIs are not available as OTC in Japan. GerdQ is a patient-centered self-assessment questionnaire that is used for the diagnosis and management of patients with GERD[12] and was previously validated in Japanese patients.[12] The cut-off score of GerdQ was set at 8 according to the previous studies.[12,13] When the GerdQ was developed as an exploratory part of the DIAMOND study, a cut-off of 8 showed the highest specificity (71.4%) and sensitivity (64.6%) for GERD.[13] Our previous study also showed that a GerdQ cut-off of 8 gave the best balance with regard to sensitivity and specificity for RE in Japanese populations.[12] Participants were excluded if they were unemployed, under treatment for any cancer or peptic ulcer, had a GerdQ score <8 despite not using medication, refused to provide informed consent, withdrew before the main survey was completed, or had a history of upper GI surgery.

The loss of work productivity, absenteeism, presenteeism, and productivity in regular daily activities were determined using the WPAI-GERD.[5] The annual income of the Japanese GERD participants was estimated from the Basic Survey on Wage Structure in 2011 (national) by the MHLW, based on the participants' employment type and age. Based on the estimated annual income for each participant, economic loss was also estimated using the WPAI-GERD.

Statistical analysis

The participants were categorized into two groups: GerdQ <8 (GERD symptoms relieved with medications) and GerdQ ≥8 (GERD symptoms persistent despite medication). Baseline characteristics were compared between the two groups using Fisher's exact test for categorical variables or Student's *t*-test for continuous variables. Loss of work productivity, absenteeism, presenteeism, and economic loss were expressed as mean ± SD values. Productivities in regular daily activities (%) were expressed as mean (the 95% confidence interval levels). Student's *t*-test was used to determine the differences in work and daily productivities between the two groups. One-way ANOVA and Tukey's *post hoc* analysis were used to determine the differences in work productivities between more than two groups. Pearson's correlation analysis was used to evaluate the association between the treatment satisfaction and loss of work productivity due to GERD. A $p < 0.05$ was considered statistically significant.

RESULTS

Participant characteristics

A total of 20 000 individuals were invited to complete the survey between 4–10 October 2012 (Fig. 1). 17 742 individuals did not meet the inclusion criteria (7156 due to unemployment, 8696 with no GERD symptoms without medications, 1685 with GerdQ scores <8 without medications, and 205 under treatment for neoplasm or peptic ulcer diseases) and 492 refused consent. As such, 1766 proceeded to the main survey, among whom 721 answered the main survey, while the other 1045 did not. After that process, eight were excluded for a history of upper GI tract surgery and 63 incompletely answered the questionnaire. Ultimately, 650 participants were enrolled in the analysis.

Among the 650 participants, 501 were identified as having persistent GERD symptoms based on GerdQ scores with or without medications and 149 had GERD symptoms that were alleviated with medications. The participants' demographic characteristics are shown in Table 1. More men than women were enrolled (428/222). A significantly greater proportion of men had persistent GERD symptoms than the proportion whose symptoms were relieved ($p = 0.02$). OTC drugs were more often used in the symptom-relieved GERD group ($p < 0.001$). The commonly used OTC drugs were traditional Japanese herbal medicines (44%), a combination of antiacids and digestive enzymes (37%), and low dose Histamine-$_2$ receptor antagonists (H_2RA)

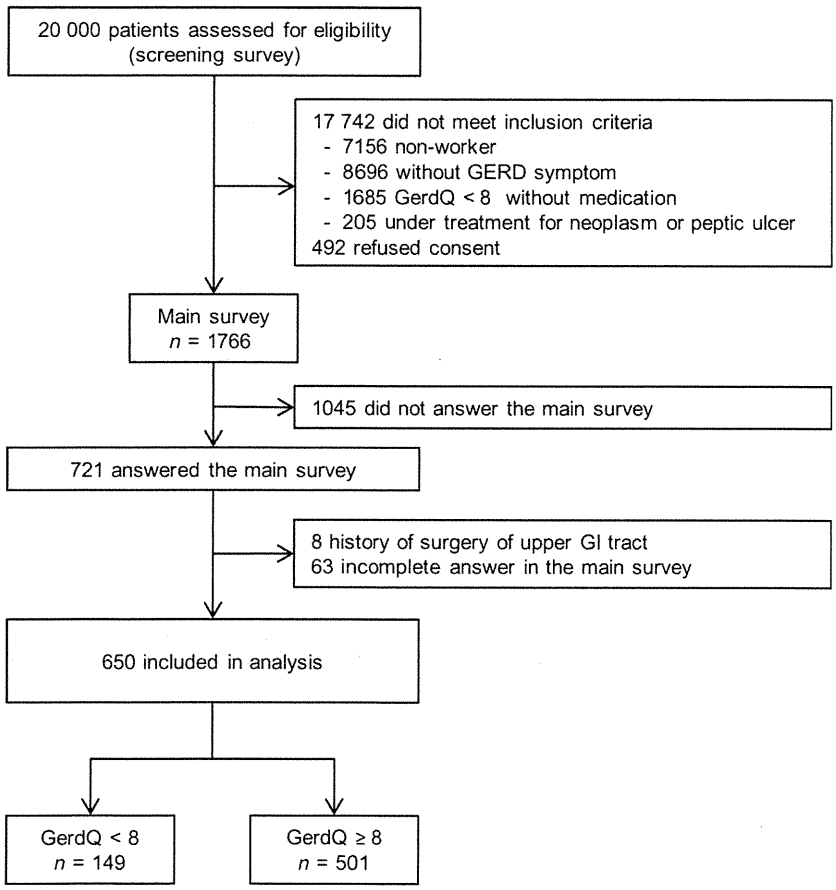

Figure 1 Patient recruitment flowchart.

Table 1 Participant demographics

	GerdQ < 8 (*n* = 149)	GerdQ ≥ 8 (*n* = 501)	*p* value
Age (years) (mean ± SD)	43.6 ± 12.1	44.4 ± 11.6	0.43†
Gender			
Men	86 (57.7%)	342 (68.3%)	**0.02**‡
Women	63 (42.3%)	159 (31.7%)	
BMI (kg/m^2) (mean ± SD)	22.5 ± 4.5	23.2 ± 4.1	0.06†
Monthly salary (,000 JPY) (mean ± SD)	276 ± 104	293 ± 105	0.10†
Medication for GERD			
PPI (prescribed)	28 (18.8%)	85 (17.0%)	0.62‡
H_2RA (prescribed)	39 (26.2%)	98 (19.6%)	0.09‡
OTC	107 (71.8%)	145 (28.9%)	**<0.001**‡
Concurrent medical history			
Diabetes mellitus	12 (8.1%)	28 (5.6%)	0.33‡
Hypertension	26 (17.4%)	71 (14.2%)	0.36‡
Dyslipidemia	14 (9.4%)	54 (10.8%)	0.76‡
Asthma	3 (2.0%)	21 (4.2%)	0.32‡
Hives	3 (2.0%)	21 (4.2%)	0.32‡
Allergic rhinitis	14 (9.4%)	56 (11.2%)	0.65‡
Hay fever	20 (13.4%)	102 (20.4%)	0.06‡
Atopic dermatitis	7 (4.7%)	20 (4.0%)	0.65‡
Rheumatic disorders	1 (0.7%)	2 (0.4%)	0.54‡

Bold values indicate significant difference. BMI, body mass index: PPI, proton pump inhibitors; H_2RA, histamine 2 receptor antagonist; OTC, over-the-counter drugs. †Student's *t*-test. ‡Fisher's exact test.

(13%). There were no significant differences between participants who had persistent *vs* alleviated GERD symptoms in mean age, body mass index, monthly salary, proportion of proton pump inhibitor (PPI) prescriptions, H_2RA prescriptions, or medical complications such as diabetes mellitus, hypertension, dyslipidemia, asthma, hives, allergic rhinitis, hay fever, atopic dermatitis, or rheumatic disorders.

Loss of productivity because of GERD

The participants with persistent GERD symptoms reported a significantly greater loss of work productivity of 11.4 ± 13.4 h/week, absenteeism of 0.7 ± 3.1 h/week, and presenteeism of 10.7 ± 12.6 h/week compared with the participants whose GERD symptoms were alleviated with medications (7.1 ± 9.5 h/week, 0.3 ± 1.4 h/week, and 6.9 ± 9.3 h/week, respectively). The resulting economic loss was significantly higher in the participants with persistent GERD symptoms (20 100 ± 26 800 JPY/week; estimated as 148.9 ± 198.5 €/week) than in the participants whose GERD symptoms were alleviated with medication (10 900 ± 15 700 JPY/week; 80.7 ± 116.3 €/week; $p < 0.001$). In addition, productivity in regular daily activities was lower in participants with persistent GERD symptoms (71.3 ± 27.0%) than in those whose symptoms were relieved (78.5 ± 25.0%, $p < 0.01$; Table 2).

Table 2 Loss of work productivity due to GERD

	GerdQ < 8 (*n* = 149)	GerdQ ≥ 8 (*n* = 501)	*p* value†
Loss of work productivity (h/week)	7.1 ± 9.5	11.4 ± 13.7	<0.001
Absenteeism (h/week)	0.3 ± 1.4	0.7 ± 3.1	0.01
Presenteeism (h/week)	6.9 ± 9.3	10.7 ± 12.6	<0.001
Economic loss (,000 JPY/week)	10.9 ± 15.7	20.1 ± 26.8	<0.001
Productivity in regular daily activities (%)	78.5 (74.2–82.7)	71.3 (69.0–73.7)	0.003

†Student's *t*-test.

Influence of comorbidities on GERD-induced productivity losses

The cohort was further characterized based on comorbidities such as diabetes mellitus and allergy (hives, allergic rhinitis, hay fever, atopic dermatitis, and rheumatic disorders; Table 3). Significantly greater absenteeism and presenteeism accompanied by loss of work productivity, economic loss, and lower productivity in regular daily activities were seen in the participants with allergies compared to those without allergies among the participants with persistent GERD symptoms despite medical therapy. Conversely, no significant differences in productivity were seen between the participants with and without allergy among participants whose GERD symptoms were alleviated with medication, nor between participants with and without diabetes mellitus (Table 3).

Influence of GERD medication on loss of productivity due to GERD

Individuals with persistent symptoms despite any medications to treat GERD (PPIs, H_2RA, or OTC) demonstrated significantly greater loss of work productivity, absenteeism, presenteeism, and economic loss, and had lower productivity in regular daily activities than individuals not taking medications (Table 4). No significant differences in productivity were found between individuals taking PPIs and those not taking PPIs within the subgroups of alleviated or persistent GERD symptoms.

Regarding H_2RA, only daily productivity was significantly lower in the patients prescribed H_2RA than in those free of H_2RA among individuals who had persistent GERD symptoms. On the other hand, as for the usage of OTCs, significantly greater loss of work productivity, absenteeism, presenteeism, economic loss, and lower productivity in regular daily activities

Table 3 Influence of concomitant diseases on the loss of work productivity due to GERD

	Loss of work productivity (h/week)	Absenteeism (h/week)	Presenteeism (h/week)	Economic loss (,000 JPY/ week)	Productivity in regular daily activities (%)
GerdQ < 8 (n = 149)					
Diabetes (−) (n = 137)	7.2 ± 9.7	0.3 ± 1.4	6.9 ± 9.5	10.8 ± 15.9	78.3 (73.8–82.7)
Diabetes (+) (n = 12)	6.7 ± 7.0	0.2 ± 0.4	6.5 ± 6.8	12.4 ± 14.7	80.8 (65.7–95.9)
p value†	0.86	0.84	0.88	0.74	0.60
Allergy (−) (n = 114)	7.1 ± 9.7	0.3 ± 1.5	6.8 ± 9.4	10.9 ± 16.3	78.4 (73.6–83.3)
Allergy (+) (n = 35)	7.4 ± 9.3	0.2 ± 0.7	7.2 ± 9.1	11.0 ± 13.8	78.6 (69.9–87.3)
p value†	0.86	0.94	0.85	0.99	0.98
GerdQ ≥ 8 (n = 501)					
Diabetes (−) (n = 473)	11.3 ± 13.6	0.7 ± 3.1	10.6 ± 12.6	19.4 ± 25.5	71.5 (69.1–73.9)
Diabetes (+) (n = 28)	13.1 ± 14.3	1.0 ± 2.9	12.1 ± 13.3	30.7 ± 42.2	67.9 (58.0–77.7)
p value†	0.49	0.56	0.55	0.17	0.48
Allergy (−) (n = 357)	10.2 ± 13.3	0.5 ± 2.7	9.7 ± 12.7	18.4 ± 26.7	74.5 (71.8–77.2)
Allergy (+) (n = 144)	14.2 ± 14.2	1.2 ± 3.9	13.0 ± 12.4	24.2 ± 26.7	63.5 (59.2–67.8)
p value†	**0.003**	**0.048**	**0.008**	**0.03**	**<0.001**

'Allergy' includes hives, allergic rhinitis, hay fever, atopic dermatitis, and rheumatic disorders. Bold values indicate significant difference. †Student's t-test.

Table 4 Medication for GERD and loss of work productivity

	Loss of work productivity (h/week)	Absenteeism (h/week)	Presenteeism (h/week)	Economic loss (,000 JPY/ week)	Productivity in regular daily activities (%)
GerdQ < 8 (n = 149)					
PPI (−) (n = 121)	7.4 ± 9.9	0.3 ± 1.5	7.2 ± 9.7	11.3 ± 16.4	77.4 (72.6–82.1)
PPI (+) (n = 28)	5.9 ± 7.7	0.2 ± 0.6	5.7 ± 7.7	9.5 ± 12.8	83.2 (73.4–93.1)
p value†	0.43	0.89	0.43	0.60	0.19
H_2RA (−) (n = 110)	7.0 ± 9.6	0.1 ± 0.4	6.9 ± 9.5	10.8 ± 16.6	79.9 (75.0–84.9)
H_2RA (+) (n = 39)	7.6 ± 9.6	0.7 ± 2.6	6.9 ± 8.7	11.4 ± 13.3	74.4 (66.0–82.7)
p value†	0.72	0.14	0.99	0.83	0.24
OTC (−) (n = 42)	6.8 ± 8.7	0.4 ± 2.2	6.4 ± 8.1	10.1 ± 12.7	78.8 (70.8–86.8)
OTC (+) (n = 107)	7.3 ± 9.9	0.2 ± 0.9	7.1 ± 9.8	11.3 ± 16.8	78.3 (73.3–83.3)
p value†	0.81	0.38	0.71	0.69	0.92
GerdQ ≥ 8 (n = 501)					
Medication‡ (−) (n = 228)	8.9 ± 12.2	0.4 ± 1.6	8.5 ± 11.8	16.2 ± 25.4	76.4 (72.9–79.8)
Medication‡ (+) (n = 273)	13.4 ± 14.5	1.0 ± 4.0	12.5 ± 13.1	23.3 ± 27.5	67.1 (64.0–70.3)
p value†	**<0.001**	**0.03**	**<0.001**	**0.003**	**<0.001**
PPI (−) (n = 416)	10.9 ± 13.2	0.5 ± 1.8	10.4 ± 12.8	19.0 ± 26.4	72.1 (69.5–74.6)
PPI (+) (n = 85)	13.8 ± 15.5	1.7 ± 6.4	12.1 ± 12.0	25.0 ± 28.2	67.8 (62.1–73.4)
p value†	0.73	0.10	0.23	0.06	0.18
H_2RA (−) (n = 403)	10.9 ± 13.8	0.7 ± 3.3	10.2 ± 12.6	19.3 ± 26.5	72.9 (70.3–75.5)
H_2RA (+) (n = 98)	13.2 ± 13.2	0.7 ± 2.3	12.5 ± 12.6	23.0 ± 28.0	64.9 (59.7–70.1)
p value†	0.13	0.94	0.10	0.22	**0.01**
OTC (−) (n = 356)	10.0 ± 12.2	0.5 ± 1.8	9.5 ± 11.8	18.0 ± 25.2	73.3 (70.6–76.1)
OTC (+) (n = 145)	14.7 ± 16.3	1.3 ± 5.0	13.4 ± 14.3	25.2 ± 29.8	66.4 (62.1–70.7)
p value†	**0.002**	**0.047**	**0.005**	**0.01**	**0.01**

Bold values indicate significant difference. PPI, proton pump inhibitors; H_2RA, histamine 2 receptor antagonists; OTC, over-the-counter drugs. †Student's t-test. ‡'Medication' indicates PPI, H_2RA, or OTC.

was observed in OTC drug users than in non-users among individuals with GERD symptoms (Table 4) while no significant differences in productivity were found between OTC users and non-users among individuals without GERD symptoms.

Among individuals with GERD symptoms, loss of work productivity and presenteeism were significantly higher in the patients taking both PPI and OTC than in those without any medications or taking PPI alone (Fig. 2A and C). In terms of absenteeism, individuals taking both PPI and OTC showed higher values than those without any medications, those taking OTC alone, or those taking PPI alone ($p < 0.001$; Fig. 2B). In terms of economic loss, individuals taking both PPI and OTC showed higher values than those taking no medications ($p < 0.01$; Fig. 2D).

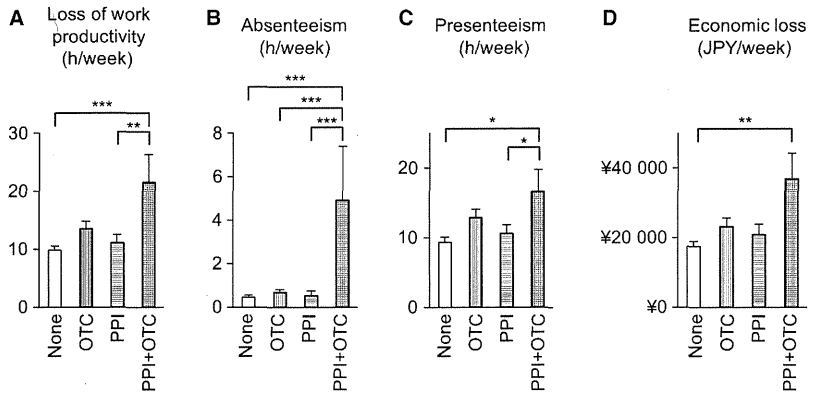

Figure 2 Loss of work productivity (A), absenteeism (B), presenteeism (C), and economic loss (D) among the patients with symptom-positive gastro-esophageal reflux disease (GERD) according to medication patterns such as non-medication, over-the-counter (OTC) medication alone, proton pump inhibitor (PPI) medication alone, and PPI+OTC medications. Statistical differences between the four groups were analyzed using one-way ANOVA and Tukey's *post hoc* analysis. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Impact of treatment dissatisfaction on loss of productivity

Among individuals in whom GERD symptoms were alleviated, treatment dissatisfaction was significantly correlated with loss of work productivity ($p = 0.048$), presenteeism ($p = 0.03$), and productivity in regular daily activities ($p = 0.02$). Among patients with persistent GERD symptoms, dissatisfaction with GERD treatment was significantly correlated with loss of work productivity ($p < 0.001$), presenteeism ($p < 0.001$), economic loss ($p < 0.001$), and productivity in regular daily activities ($p = 0.02$; Table 5).

DISCUSSION

Using a Web-based survey to quantify the extent of decrease in work and daily productivity associated with GERD symptoms in 650 Japanese workers, this study revealed that persistent GERD symptoms, with or without medications, significantly reduced work and daily productivities of Japanese workers and resulted in severe economic loss, as compared with in whom GERD symptoms were alleviated.

In this study, individuals with persistent GERD symptoms showed a higher loss of work and daily productivity than individuals in whom a PPI, H_2RA, or OTC medication alleviated their symptoms, confirming that symptom control by medications could be beneficial for decreasing the working and daily burden due to GERD. Individuals with persistent GERD symptoms who were taking OTC in addition to prescribed PPI medications showed the highest loss of productivity and its related economics, suggesting that incomplete doses of PPI medications would lead not only to OTC consumption but also to severe productivity and economic losses. In other words, patients with symptoms in spite of PPI use are likely to suffer from more severe disease than those using less potent medication.

Influence of comorbid disease on the loss of work and daily productivities was also evaluated. Although diabetes mellitus had no significant influence on

Table 5 Treatment satisfaction and loss of work productivity due to GERD

	Loss of work productivity (h/week)	Absenteeism (h/week)	Presenteeism (h/week)	Economic loss (,000 JPY/week)	Productivity in regular daily activities (%)
GerdQ < 8 ($n = 149$)					
Very satisfied ($n = 10$)	4.5 ± 8.4	0.1 ± 0.3	4.4 ± 8.3	7.8 ± 13.1	84.0 (67.6–82.7)
Satisfied ($n = 71$)	6.5 ± 9.2	0.4 ± 1.9	6.1 ± 8.8	10.2 ± 15.5	81.7 (74.2–100)
Neither satisfied nor dissatisfied ($n = 57$)	7.2 ± 9.6	0.1 ± 0.3	7.2 ± 9.6	10.7 ± 13.0	77.0 (70.2–83.9)
Dissatisfied ($n = 8$)	14.5 ± 11.2	0.4 ± 1.1	14.1 ± 10.4	19.8 ± 14.4	53.8 (35.4–72.1)
Very dissatisfied ($n = 3$)	10.7 ± 13.0	0.0 ± 0.0	10.7 ± 13.0	18.0 ± 21.2	76.7 (46.8–107)
p value (for trend)†	**0.048**	0.46	**0.03**	0.13	**0.02**
GerdQ ≥ 8 ($n = 192$)					
Very satisfied ($n = 10$)	5.9 ± 9.6	0.6 ± 1.3	5.3 ± 8.5	11.2 ± 18.7	76.0 (59.6–92.4)
Satisfied ($n = 82$)	9.7 ± 11.7	0.4 ± 1.3	9.3 ± 11.3	17.0 ± 25.7	71.8 (66.1–77.5)
Neither satisfied nor dissatisfied ($n = 69$)	16.6 ± 15.8	1.8 ± 5.6	14.8 ± 13.3	29.4 ± 28.6	65.2 (59.0–71.4)
Dissatisfied ($n = 25$)	17.2 ± 19.0	2.4 ± 8.1	14.8 ± 16.2	28.1 ± 33.1	56.0 (45.6–66.4)
Very dissatisfied ($n = 6$)	33.6 ± 11.5	0.0 ± 0.0	33.6 ± 11.5	49.6 ± 16.2	33.3 (12.2–54.5)
p value (for trend)†	**<0.001**	0.11	**<0.001**	**<0.001**	**<0.001**

Bold values indicate significant difference. †Pearson's correlation test.

productivity, hay fever and other allergic disorders worsened the productivity and economic losses in individuals with persistent GERD symptoms regardless of the use of medications, suggesting the synergistic effects of symptomatic disorders on work and daily productivities.

In this study, among the participants with GERD symptoms, absenteeism accounted for a mean loss of work productivity of 0.7 h/week, while presenteeism accounted for a loss of 10.7 h/week. Thus, GERD symptoms generally did not prevent workers from attending work but did reduce their work productivity by 26.9%. According to a systematic review of nine studies, the loss of work productivity due to presenteeism was 5.2–39.7%,[7] which is in line with our findings in Japanese workers. This is consistent with results of previously performed studies in Western countries.[5–10]

However, compared with workers in the United States, where presenteeism resulted in a loss of work productivity of 6–17%,[6,9] loss of work productivity due to presenteeism is markedly higher in Japanese workers with GERD. In line with the earlier study reporting association between the loss of work productivity associated with presenteeism and the severity of GERD symptoms,[8] the present results clearly show a higher loss of work and daily productivity and economic costs in patients with GerdQ scores ≥8 than in those with GerdQ scores <8.

A number of different medications, including prescription and OTC drugs, were taken by the participants of our Web survey. It was clearly shown that the decrease in work and daily productivity of the Japanese workers with symptom-positive GERD was significantly correlated with dissatisfaction with GERD treatment, indicating that many treatments for GERD that are currently used in Japan provide limited symptom relief and do not always completely prevent loss of productivity in work or daily life and the associated economic loss.

This study had several limitations. Firstly, as the enrolled participants were derived from those registered in a database of the survey Web site, non-Internet users, especially elderly individuals, may not have been included in the database or available for this study. The mean age for the whole present analyzed participants was 44 years. Secondly, the participants were not diagnosed with GERD by physicians. However, as the use of the Japanese version of GerdQ to determine GERD symptoms was validated in our earlier study[12] and in this study the presence of GERD was defined as a GerdQ sum score ≥8, as previously validated,[12] it was considered that GERD was properly diagnosed based on the symptomatic measurement by the validated questionnaire. On the other hand, the symptom-relieved patients on medication were diagnosed with GERD based on a GerdQ score <8, which usually indicates low likelihood of GERD.

In conclusion, persistent GERD symptoms despite medical therapy place a significantly higher burden on work and daily productivities and economic costs than symptom-controlled GERD. Relief of GERD symptoms by any medications could reduce the loss of work and daily productivities and economic loss, whereas insufficient treatments for GERD may be supposed to worsen productivity and economic losses.

FUNDING

This study was supported by the grants from AstraZeneca KK, Osaka, Japan and from the Smoking Research Foundation (to HS).

DISCLOSURE

During the last 2 years, Hidekazu Suzuki received scholarship funds for the research from Astellas Pharm Inc., Astra-Zeneca KK, Otsuka Pharmaceutical Co., Ltd., and Takeda Pharmaceutical Co., Ltd., and Zeria Pharmaceutical Co., Ltd.

AUTHOR CONTRIBUTION

HS designed the study; HS, JM, and TM collected and interpreted the data; HS and JM performed the statistical analysis; HS drafted the article; JI revised the draft critically; All of the authors approved the final version of the article.

REFERENCES

1 Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am J Gastroenterol* 2006; **101**: 1900–20.
2 Hongo M, Kinoshita Y, Miwa H, Ashida K. The demographic characteristics and health-related quality of life in a large cohort of reflux esophagitis patients in Japan with reference to the effect of lansoprazole: the REQUEST study. *J Gastroenterol* 2008; **43**: 920–7.
3 Yoshida S, Nii M, Date M. Effects of omeprazole on symptoms and quality of life in Japanese patients with reflux esophagitis: final results of OMAREE, a large-scale clinical experience investigation. *BMC Gastroenterol.* 2011; **11**: 15.
4 Kulig M, Leodolter A, Vieth M, Schulte E, Jaspersen D, Labenz J, Lind T, Meyer-Sabellek W *et al.* Quality of life in relation to symptoms in patients with gastro-oesophageal reflux disease–an analysis based on the ProGERD initiative. *Aliment Pharmacol Ther* 2003; **18**: 767–76.
5 Wahlqvist P, Carlsson J, Stalhammar NO, Wiklund I. Validity of a Work Productivity and Activity Impairment

questionnaire for patients with symptoms of gastro-esophageal reflux disease (WPAI-GERD)–results from a cross-sectional study. *Value Health.* 2002; **5**: 106–13.
6 Dean BB, Crawley JA, Schmitt CM, Wong J, Ofman JJ. The burden of illness of gastro-oesophageal reflux disease: impact on work productivity. *Aliment Pharmacol Ther* 2003; **17**: 1309–17.
7 Wahlqvist P, Reilly MC, Barkun A. Systematic review: the impact of gastro-oesophageal reflux disease on work productivity. *Aliment Pharmacol Ther* 2006; **24**: 259–72.
8 Wahlqvist P, Karlsson M, Johnson D, Carlsson J, Bolge SC, Wallander MA. Relationship between symptom load of gastro-oesophageal reflux disease and health-related quality of life, work productivity, resource utilization and concomitant diseases: survey of a US cohort. *Aliment Pharmacol Ther* 2008; **27**: 960–70.
9 Dubois RW, Aguilar D, Fass R, Orr WC, Elfant AB, Dean BB, Harper AS, Yu HT *et al.* Consequences of frequent nocturnal gastro-oesophageal reflux disease among employed adults: symptom severity, quality of life and work productivity. *Aliment Pharmacol Ther* 2007; **25**: 487–500.
10 Van Oudenhove L, Geeraerts B, Tack J. Limitations of current paradigms for visceral sensitivity testing. *Neurogastroenterol Motil* 2008; **20**: 95–8.
11 Gisbert JP, Cooper A, Karagiannis D, Hatlebakk J, Agreus L, Jablonowski H, Nuevo J. Impact of gastroesophageal reflux disease on work absenteeism, presenteeism and productivity in daily life: a European observational study. *Health Qual Life Outcomes.* 2009; **7**: 90.
12 Suzuki H, Matsuzaki J, Okada S, Hirata K, Fukuhara S, Hibi T. Validation of the GerdQ questionnaire for the management of gastro-oesophageal reflux disease in Japan. *United European Gastroenterology Journal.* 2013; **1**: 175–83.
13 Jones R, Junghard O, Dent J, Vakil N, Halling K, Wernersson B, Lind T. Development of the GerdQ, a tool for the diagnosis and management of gastro-oesophageal reflux disease in primary care. *Aliment Pharmacol Ther* 2009; **30**: 1030–8.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Key elements of the Work Productivity and Activity Impairment questionnaire for gastro-oesophageal reflux disease (WPAI-GERD).