J Exp Clin Med 2014;6(3):98–101

Contents lists available at ScienceDirect

Journal of Experimental and Clinical Medicine

journal homepage: http://www.jecm-online.com

SHORT COMMUNICATION

Erlotinib: Lacking of Cholinergic Effects on Tracheal Smooth Muscle

Chih-Cheng Chang [1,2,3], Hsiao-Chi Chuang [2,3], Chun-Nin Lee [2,3], Wen-Yueh Hung [3], Hsing-Won Wang [1,4,5*]

[1] Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[2] School of Respiratory Therapy, College of Medicine, Taipei Medical University, Taipei, Taiwan
[3] Division of Pulmonary Medicine, Department of Internal Medicine, Shuang Ho Hospital, Taipei Medical University, Taipei, Taiwan
[4] Department of Otolaryngology, Shuang Ho Hospital, Taipei Medical University, Taipei, Taiwan
[5] Department of Preventive and Community Medicine, Shuang Ho Hospital, Taipei Medical University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received: Feb 13, 2014
Revised: Feb 17, 2014
Accepted: Mar 20, 2014

KEY WORDS:
cholinergic effect;
electrical field stimulation;
erlotinib;
tracheal smooth muscle

Erlotinib (Tarceva) is an oral epidermal growth factor receptor-tyrosine kinase inhibitor that is mainly used for patients with advanced or metastatic non-small-cell lung cancer. Tyrosine kinase signaling cascades also play a critical role in the pathogenesis of allergic airway inflammation and airway remodeling. However, cholinergic effects caused by erlotinib on tracheal smooth muscle remain unclear. The objective of this study was to determine the effects of erlotinib on the isolated rat tracheal smooth muscle *in vitro*. To examine the cholinergic effects of erlotinib, *in vitro* rat tracheal smooth muscle was used to assess alterations in methacholine-induced contraction (served as a parasympathetic mimetic) and electrically induced contraction. The results demonstrated that the addition of erlotinib (from 1×10^{-8}M to 1×10^{-4}M) induced no significant effects on tracheal tension after methacholine treatment. Furthermore, erlotinib did not affect electrical field stimulation-induced spike contraction. This study demonstrated that erlotinib had no cholinergic effects *in vitro*, suggesting it may be safe for asthmatic patients with non-small-cell lung cancer after further investigation.

1. Introduction

Lung cancer is a leading cause of cancer-related deaths worldwide, and its incidence has been increasing. Non-small-cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancers. Patients with NSCLC harboring mutations in the epidermal growth factor receptor (EGFR) gene have a dramatic response to EGFR-tyrosine kinase inhibitor (EGFR-TKI).[1] Therapeutic modalities used in thoracic oncology include molecularly targeted therapy using low-molecular-weight TKIs that block the activation of the EGFR cascade. Erlotinib (Tarceva) is an oral EGFR-TKI that is mainly used for patients with advanced or metastatic NSCLC who have failed at least one prior chemotherapy regimen. First-line treatment with EGFR-TKIs such as erlotinib showed higher efficiency than standard chemotherapy regimens in patients harboring EGFR mutations.[2,3]

Asthma is a very common chronic disease that occurs in all age groups. Association between asthma and lung cancer has been reported,[4] suggesting that asthma is a risk factor of lung cancer development. However, there is a paucity of studies evaluating the risk of lung cancer treatment in patients with asthma. Comparisons of the efficacy and safety of erlotinib with standard chemotherapy regimens for second-line therapy confirmed that erlotinib has comparable efficiency and a better toxicity profile.[5,6] However, TK signaling cascades play a critical role in the pathogenesis of allergic airway inflammation. Receptor TKs such as EGFR are important for the pathogenesis of airway remodeling.[7] It has been demonstrated that EGFR expression is increased in asthmatic human airway.[8] In human airway smooth muscle cells, both epidermal and platelet-derived growth factors have been revealed to promote EGFR and platelet-derived growth factor receptor tyrosine autophosphorylation, leading to transcription factor activation and proliferation.[9] However, effects of EGFR signaling on allergic responses induced by erlotinib remain unclear. Using trachea isolated from rats we have developed a simple *in vitro* model to study agents that affect tracheal smooth muscle.[10] This system can provide more evidence of the cholinergic effects in response of the trachea to drugs *in vivo*. To clarify this issue, we used tracheal smooth muscle *in vitro*, which

Conflicts of interest: The authors declare no conflicts of interest.

* Corresponding author. Hsing-Won Wang, Graduate Institute of Clinical Medicine and Department of Otolaryngology, School of Medicine, Taipei Medical University—Shuang Ho Hospital, Number 291, Jhong-Jheng Road, Jhongghe District, New Taipei City, Taiwan.
E-mail: H.-W. Wang <w0512n@ms15.hinet.net>

http://dx.doi.org/10.1016/j.jecm.2014.03.007

has an important role in the response to asthma attacks, to examine the cholinergic effects of erlotinib.

2. Methods

2.1. Reagent sources

This study was approved by the Animal Research Committee of Taipei Medical University (LAC-99-0299; Taipei, Taiwan). Pure erlotinib was obtained from the Roche Company (Taipei, Taiwan). All other reagents were obtained from Sigma-Aldrich (St. Louis, Missouri, USA).

2.2. Tissue sampling and preparation

We obtained 18 8-week-old Sprague—Dawley rats from the National Laboratory Animal Breeding and Research Centre (Taipei, Taiwan). Rats were anesthetized via intraperitoneal pentobarbital injections (45 mg/kg), and two 5-mm long pieces of trachea were obtained from each rat; this procedure had been described in detail in previous studies.[11,12] The upper side of the tracheal sample was attached to a Grass FT-03 force displacement transducer (AstroMed, West Warwick, RI, USA) using a steel plate and a 3-0 silk ligature, whereas the other side was fixed to a steel plate attached to a container with 30 mL of Kerb's solution (NaCl, 118 mmol/L; KCl, 4.7 mmol/L; $CaCl_2$, 2.5 mmol/L; $MgSO_4 \cdot 7H_2O$, 1.2 mmol/L; KH_2PO_4, 1.2 mmol/L; $NaHCO_3$, 25.0 mmol/L; and glucose, 10.0 mmol/L) at 37°C. A passive tension of 0.3 g was applied to the strips, and subsequent changes in tension were recorded continuously using Chart V4.2 software (PowerLad; ADI Instruments, Colorado Springs, CO, USA).

2.3. Methacholine challenge

Methacholine (1×10^{-6}M) was used as a tracheal contractor in this study. A preliminary experiment was performed using a tracheal strip to determine the contraction when basal tension was applied. Isolated tracheas were equilibrated in the solution for 30 minutes and aerated with a mixture of 95% O_2 and 5% CO_2. Stepwise increases in erlotinib concentrations (from 1×10^{-8}M to 1×10^{-4}M; dissolved in d-H_2O) were used to investigate the contraction or relaxation responses of the tracheal strips. All treatments were administrated by adding a defined volume of stock solution to the solution. After the experiment, 1×10^{-4}M lidocaine was used to reduce the tension caused by methacholine and/or erlotinib.

2.4. Electrical field stimulation challenge

Electrical field stimulation (EFS; 5 Hz, 5-millsecond pulse duration, voltage of 50 V, stimulation trains of 5-second duration) was applied to the tracheal strip with two wire electrodes placed parallel to the strip and connected to a direct-current stimulator (Grass S44, Grass Instruments, Quincy, MA, USA). A 2-minute interval was imposed between each stimulation period to allow recovery from the response. The stimulation experiment was performed at 37°C.

2.5. Measurements

The effects of erlotinib on tracheal smooth muscle resting tension and 1×10^{-6}M methacholine and erlotinib on electrically induced tracheal smooth muscle contractions were determined. In each experiment, one untreated tracheal strip served as a control. Concentrations of erlotinib were expressed as the concentrations present in the 30-mL solution.

2.6. Statistical analysis

Data for the basal tension and methacholine experiments were presented as the mean tension induced by two different concentrations of the agent. EFS data were presented as the mean EFS peak induced by two different concentrations of the agent. Student *t* test was used to evaluate the differences. All statistical analyses in this study were performed with SPSS 15.0 software (SPSS Inc., Chicago, Illinois, USA). The level of significance for all statistical analyses was $p < 0.05$. Data were presented as the mean ± standard deviation.

3. Results

Tracheal responses to the treatments were determined from the tension applied to the transducer. Control experiments were initially performed to measure the tracheal contraction induced by 1×10^{-6}M methacholine (Figure 1). We then treated the tissue with increasing concentrations (from 1×10^{-8}M to 1×10^{-4}M) of erlotinib and determined the alterations in contraction/relaxation after treatment (Figure 1). A slight decrease was observed in the contractile responses to erlotinib exposure, albeit without significance. The percentages of contraction in the tracheal tissues were 99.0 ± 1.1 (at 1×10^{-8}M erlotinib), 98.4 ± 1.4 (at 1×10^{-7}M erlotinib), 97.8 ± 1.5 (at 1×10^{-6}M erlotinib), 97.3 ± 2.0 (at 1×10^{-5}M erlotinib), and 97.0 ± 1.8 (at 1×10^{-4}M erlotinib), as shown in Figure 2. A relaxant drug (1×10^{-4}M lidocaine) was applied to the tissue to examine the reaction of the tracheal strips.

The effects of erlotinib on electrically induced tracheal smooth muscle contraction were examined. The results revealed that no significant EFS response was induced by the addition of different concentrations of erlotinib (Figure 3). Percentage changes of peak tension in the tissues were reduced slightly from 100 (control) to 98.4 ± 1.9, 98.1 ± 1.6, 97.0 ± 2.0, and 97.1 ± 1.7 at 1×10^{-7}M,

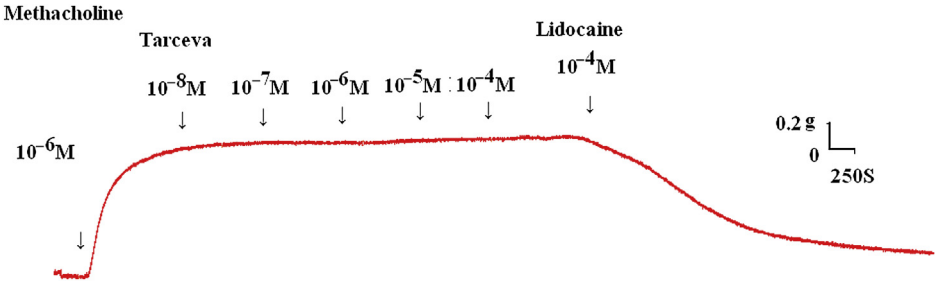

Figure 1 Original recording of the effects of erlotinib on 10^{-6}M methacholine-induced tracheal smooth muscle contractions. Tension changes in tracheal smooth muscle strip were demonstrated after treatment with 10^{-8}M and 10^{-4}M erlotinib. No significant effects were caused by erlotinib. Methacholine was initially used to induce tracheal contraction, whereas lidocaine was used to relax the tracheal muscle after the test.