Available online at www.sciencedirect.com

ScienceDirect

Journal of the Chinese Medical Association 81 (2018) 631–635

www.jcma-online.com

Original Article

Vardenafil inhibiting parasympathetic function of tracheal smooth muscle

Fei-Peng Lee [a], Pin-Zhir Chao [a], Hsing-Won Wang [a,b,c,*]

[a] *The Graduate Institute of Clinical Medicine and Department of Otolaryngology, College of Medicine, Taipei Medical University–Shuang Ho Hospital, Taipei, Taiwan, ROC*

[b] *Department of Preventive and Community Medicine, College of Medicine, Taipei Medical University–Shuang Ho Hospital, Taipei, Taiwan, ROC*

[c] *Department of Otolaryngology–Head and Neck Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, ROC*

Received September 1, 2017; accepted October 26, 2017

Abstract

Background: Levitra, a phosphodiesterase-5 (PDE5) inhibitor, is the trade name of vardenafil. Nowadays, it is applied to treatment of erectile dysfunction. PDE5 inhibitors are employed to induce dilatation of the vascular smooth muscle. The effect of Levitra on impotency is well known; however, its effect on the tracheal smooth muscle has rarely been explored. When administered for sexual symptoms via oral intake or inhalation, Levitra might affect the trachea.

Methods: This study assessed the effects of Levitra on isolated rat tracheal smooth muscle by examining its effect on resting tension of tracheal smooth muscle, contraction caused by 10^{-6} M methacholine as a parasympathetic mimetic, and electrically induced tracheal smooth muscle contractions.

Results: The results showed that adding methacholine to the incubation medium caused the trachea to contract in a dose-dependent manner. Addition of Levitra at doses of 10^{-5} M or above elicited a significant relaxation response to 10^{-6} M methacholine-induced contraction. Levitra could inhibit electrical field stimulation-induced spike contraction. It alone had minimal effect on the basal tension of the trachea as the concentration increased.

Conclusion: High concentrations of Levitra could inhibit parasympathetic function of the trachea. Levitra when administered via oral intake might reduce asthma attacks in impotent patients because it might inhibit parasympathetic function and reduce methacholine-induced contraction of the tracheal smooth muscle.

Copyright © 2018, the Chinese Medical Association. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords: In vitro study; Smooth muscle; Trachea; Vardenafil

1. Introduction

The enzyme phosphodiesterase-5 (PDE5) is known to be abundant in lung tissue where it hydrolyses cyclic guanosine monophosphate (cGMP), a second messenger of NO, causing constriction of the blood vessel walls.[1] Four PDE5 inhibitors namely sildenafil, tadalafil, vardenafil and avanafil have been clinically approved for treating erectile dysfunction (ED).[2] They are also employed to induce dilatation of the vascular smooth muscle and to inhibit platelet aggregation in treating testicular torsion, pulmonary hypertension, coronary artery disease, diabetes mellitus, chronic peripheral arterial diseases, ischemic colitis and acute mountain sickness.[3–9] There should be particular caution when prescribing PDE5 inhibitors for erectile dysfunction in patients receiving protease inhibitors. PDE5 inhibitors appear to work in men regardless of the reasons behind their erectile dysfunction, including vascular disease, nerve problems, and even psychological causes. PDE5-inhibiting drugs can have a number of side effects, including headache, dizziness,

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Hsing-Won Wang, Department of Otolaryngology, Taipei Medical University-Shuang Ho Hospital, 291, Jhong-Jheng Road, Jhonghe District, New Taipei City 235, Taiwan, ROC.

E-mail address: w0512n@ms15.hinet.net (H.-W. Wang).

https://doi.org/10.1016/j.jcma.2017.10.007

1726-4901/Copyright © 2018, the Chinese Medical Association. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

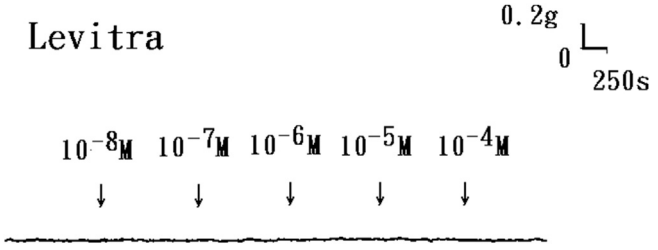

Fig. 1. Tension changes in rat trachea after application of various Levitra concentrations. Levitra alone had minimal effect on the basal tension of trachea as the concentration increased. Original basal tension was 0.3 g.

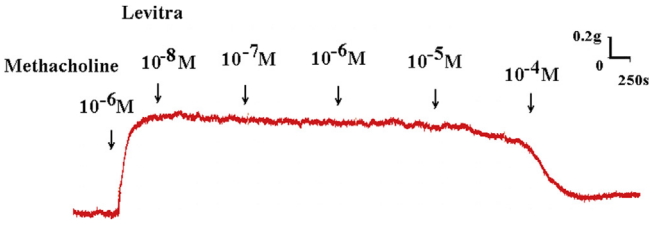

Fig. 2. Original recording of the effects of Levitra on 10^{-6} M methacholine-induced contraction of rat trachea.

lightheadedness, flushing, nasal congestion, and changes in vision.[10] (see Figs. 1–5)

Levitra is the trade name of vardenafil. It has been used as a PDE5 inhibitor to treat ED for a decade.[2] The side effects of PDE5 inhibitors had been reported but their effect on the trachea was rarely mentioned. During an asthma attack, the tracheal smooth muscle becomes contracted, thus reducing pulmonary function. A male patient with asthma may also apply this drug via oral intake or inhalation for his sexual problems. Hence, the effect of Levitra on tracheal smooth muscle merits further exploration. The aim of this study was to determine the effects of Levitra on the isolated tracheal smooth muscle *in vitro*.

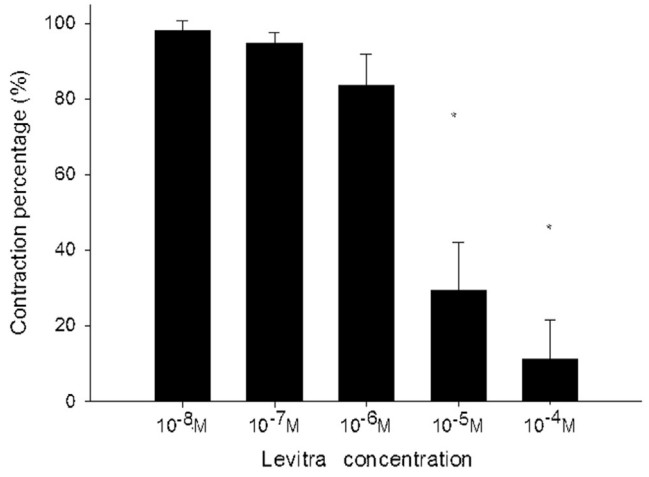

Fig. 3. Effects of Levitra on 10^{-6} M methacholine-induced contraction (contraction area calculated at 100% without addition of Levitra) of rat trachea. The difference in tension between 10^{-8} M Levitra and 10^{-5} M Levitra or 10^{-4} M Levitra was statistically significant ($p < 0.05$). Results were mean ± SD (n = 6).

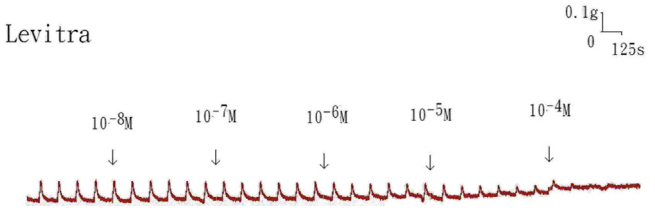

Fig. 4. Original recording of effects of Levitra on electrically induced tracheal smooth muscle contractions was noted. Higher doses of Levitra also decreased the spike contraction induced by EFS.

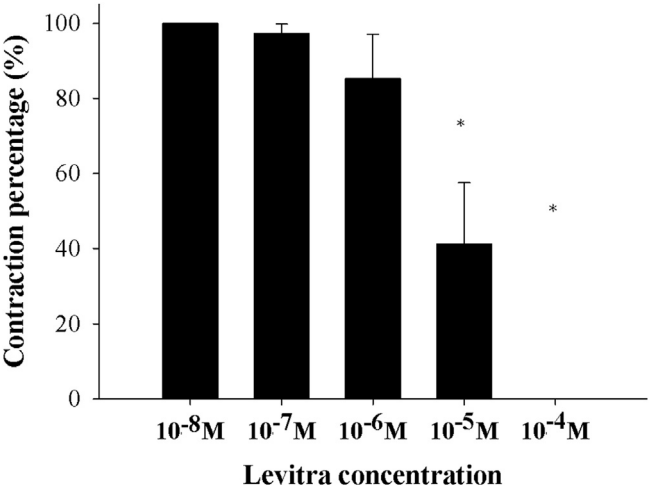

Fig. 5. Effects of Levitra on electrically induced tracheal smooth muscle contractions (contraction area calculated at 100% without addition of Levitra). The peak tension of the tracheal strip evoked by EFS during the addition of 10^{-4} M Levitra was significantly lower than that at the addition of 10^{-8} M Levitra ($p < 0.001$). Results were mean ± SD (n = 6).

2. Methods

Chemicals used were of the highest purity available. Levitra was obtained courtesy of Bayer Co., Taiwan. All other chemical reagents were obtained from Sigma (St. Louis, MO, USA). Methacholine was tested as a tracheal contraction drug. After being anesthetized by intraperitoneal administration of pentobarbital (45 mg/kg), 18 male Sprague–Dawley rats (weighing 250–300 g) were humanely killed by cervical dislocation, and two pieces of trachea (~5 mm in length) were removed from each rat. This study was approved by an animal experiment review board (IACUC-07-133). The tracheal specimen was mounted using two steel plates and submersed in a 30-mL muscle bath at 37 °C as previously reported.[11,12] Briefly, the bath was filled with 30 mL Krebs solution consisting of (mmol/L): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; and glucose, 10.0. Levitra was dissolved in dimethylsulphoxide (DMSO) and subsequently diluted in Krebs solution. Our preliminary *in vitro* studies showed that the vehicle (diluted DMSO) had no effect on the rat tracheal smooth muscle. The upper side of the

tracheal strip 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. The other side of the strip was fixed to a steel plate attached to the bath. A passive tension of 0.3 g was applied to the strips and subsequent changes in tension were continuously recorded using Chart V 4.2 software (PowerLab, AD Instruments, Colorado Springs, CO, USA). Preliminary tests showed that the tracheal strip immersed in the bath solution used for subsequent experiments did not contract when basal tension was applied. Prior to drug assays, isolated tracheas were equilibrated in the bath solution for 15–30 min, during which they were continuously aerated with a mixture of 95% O_2 and 5% CO_2. Stepwise increases in the amount of drugs used were made to study the contraction or relaxation responses of the tracheal strips. All drugs were administered by adding a defined volume of stock solution to the tissue bath solution. In each experiment, one untreated strip served as a control.

Electrical field stimulation (EFS) (5-Hz, 5-ms pulse duration, at 50 V, trains of stimulation for 5 s) was applied to the trachea strip with two wire electrodes placed parallel to the trachea strip and connected to a direct-current stimulator (Grass S44, Quincy, MA, USA). An interval of 2 min was imposed between each stimulation period to allow recovery from the response. Stimulation was continuously applied to the trachea at 37°C.

The following assessments for Levitra were made. (1) Effect on tracheal smooth muscle resting tension: this test examined the effect of the drug on the simulating condition of resting trachea condition. (2) Effect on contraction caused by 10^{-6} M methacholine (a parasympathetic mimetic): this procedure examined postsynaptic events such as muscle-receptor blockade, enhancement, and second messengers. (3) Effect of Levitra on electrically induced contractions: electrical stimulation of this tissue causes parasympathetic nerve remnants in the trachea to release a transmitter (acetylcholine). If there is interference with transmitter release, electrical stimulation does not cause contraction. Thus, presynaptic events were seen more easily with this procedure.

The concentrations of drugs were expressed as concentrations present in the 30-mL bath solution. Data were presented as Mean ± SD and statistical significance was tested using a two-tailed Student's *t*-test; p values less than 0.05 were considered significant.

3. Results

The tension applied to the transducer could indicate the degree of contraction or relaxation of the tracheal strips. Tracheal contraction induced by a small dose of methacholine was easily detected, and the tissue remained in a contracted state until the drug was rinsed from the tissue.

Addition of Levitra to the basal tension elicited a negligible effect (Fig. 1). When introduced after adding a constricting agent such as 10^{-6} M methacholine, Levitra resulted in relaxation of the trachea (Fig. 2). Low doses of Levitra mildly affected contraction while higher doses significantly relaxed the trachea smooth muscle (Figs. 2, 3). With 10^{-8} M Levitra added, the tension was 98.2 ± 2.6% of the control values (Fig. 3). At the addition of 10^{-5} M and 10^{-4} M Levitra, the tensions were 29.3 ± 12.8% and 11.2 ± 4.2%, respectively (Fig. 3). The differences in tension among the specimens treated with 10^{-8} M Levitra and 10^{-5} M or 10^{-4} M Levitra were statistically significant ($p < 0.05$).

Levitra also inhibited the spike contraction induced by EFS (Figs. 4, 5). The spike contraction was decreased with increase in Levitra concentration. The minimal increasing basal tone was not significant. The peak tension of the tracheal strip evoked by EFS with 10^{-8} M Levitra added was 100 ± 0%; whereas at the addition of 10^{-5} M and 10^{-4} M Levitra, the peaks were 41.2 ± 16.4% and 0, respectively (Fig. 5). The peak tension of the tracheal strip evoked by EFS with 10^{-5} M or 10^{-4} M Levitra added was significantly lower than that at the addition of 10^{-8} M Levitra ($p < 0.05$).

4. Discussion

PDE5 was identified as the enzyme that degraded cGMP.[1] PDE5 resides in vascular smooth muscle cells and platelets. Researchers theorized that inhibition of this enzyme offered potential benefits for patients with hypertension or angina. The initial study designed to assess the safety of PDE5 inhibitors revealed no unusual findings. In 1992, a multiple-dose phase 1 trial was initiated. A few of the study patients reported an adverse event with a tendency to get erections. The drug was tested in men with angina laterally. The hemodynamic effects were fairly mild. Rajfer et al. reported that nitric oxide (NO) caused smooth muscle relaxation in human cavernous tissue.[13] The basic mechanism involved sexual stimulation that triggered the release of NO from nerve endings in the penis. The NO in turn stimulated the production of cGMP, which dilated penile blood vessels and relaxed the smooth muscle in the walls of the cavernous sinusoids. The effects promote engorgement of the penis and penile rigidity.[14] An earlier report reviewed the effects of PDE1-5 inhibitors on airway smooth muscle *in vitro* and *in vivo*.[15] Isoenzyme-selective PDE inhibitors, especially PDE 4, might be useful airway smooth muscle relaxants in the treatment of lung disorders. Reports regarding the effects of PDE5 inhibitors on tracheal smooth muscle were scarce. This study showed that a PDE5 inhibitor such as Levitra did have effects on trachea smooth muscles.

This study was effective and simple. An intact tracheal ring, which is much more representative of a physiological setting than smooth muscle strips, was an important component of the technique involved.[11,12] It was difficult to determine which tissue component of the trachea was responsible for the drug-induced contraction, but the nature of the specific tissues and their responses to specific drugs provided some indication. The present results could be interpreted within the context of the test materials used. The tracheal strips used in this study were crude preparations containing tracheal smooth muscle and cartilage. The smooth muscle of the trachea appeared to be the main tissue component responsible for contraction. Other components such as epithelium, glands, connective

tissue, nerves, and cartilage did not contract to a significant extent. Since this method involved cross contraction, changes in tension were caused by radial contraction of the tracheal ring. Responses to drugs and electrical stimulation have been verified for similar preparations,[16−18] but the contractile response observed in this study was probably an aggregate of the responses of various types of muscle tissue. It was not easy to obtain human tissue for similar studies. The effect of this drug on isolated human tracheal smooth muscle still needs further investigation. Since this was an *in vitro* study, there were reservations as to its comparability with an *in vivo* situation in humans. In the *in vivo* situation, the response might be much more complicated than that in the *in vitro* situation.

By increasing microcirculatory blood flow, PDE5 inhibitors positively affect the oxygen uptake of tissue and are thus employed to treat patients with chronic peripheral arterial diseases and acute mountain sickness.[19] In view of the above results, Levitra might be an effective treatment option in asthmatic patients. The adverse event profiles of PDE5 inhibitors are generally similar. Class-specific side effects include headache, flushing, nasal congestion, dyspepsia and myalgia, which reflect the vasodilatory effects on the capillary smooth muscle in other parts of the body. In this study, Levitra alone had minimal effect on the basal tension of trachea as the concentration increased.

The cholinergic contracting agent tested in this preparation is commonly used for research purposes. Of note is that Levitra-induced relaxation of tissue depends on prior partial contraction of the smooth muscle after applying methacholine. Thus, it should be possible to assess the effects of common drugs and potential therapeutic agents supposedly responsible for relieving asthma attacks. Levitra, a PDE5 inhibitor, could reduce methacholine-induced contraction. Commercial oral Levitra dosage is 10 or 20 mg. The blood concentration would reach 10^{-5} M one hour after oral intake. It would undoubtedly affect the trachea. The mechanism by which Levitra affected the trachea smooth muscle is unknown and further studies are needed to elucidate the answer.

Electrical field stimulation is a common experimental tool; it activates the nerve terminals within the tissue to be tested and induces release of endogenous neurotransmitters, thereby triggering smooth muscle contraction. EFS-induced spike contraction of canine nasal mucosa, which is believed to result from the contraction of vascular smooth muscles, disappeared following ipsilateral cervical sympathetic ganglionectomy.[20] Thus, EFS-induced spike contraction of isolated canine nasal mucosa was proven to be mediated by sympathetic innervations.[20] In this study, EFS-induced spike contraction of the tracheal smooth muscle was attributed to stimulation of parasympathetic innervation. Therefore, EFS-induced contraction of the trachea decreased as the Levitra concentration increased. These findings suggested that Levitra could antagonize the parasympathetic innervation responsible for trachea smooth muscle contraction. If there is interference with transmitter release, electrical stimulation does not cause contraction. The inhibition of parasympathetic acetylcholine release by Levitra in this case was plausible. In addition, basal tension elicited minimal effect with various concentrations of Levitra. Clearly, what was observed in this study is very interesting, but further study is needed to clarify these phenomena.

The results of the present study showed that high concentrations of Levitra might actually inhibit parasympathetic function of the trachea. It could also reduce methacholine-induced contraction. In addition to ED symptoms, Levitra could effectively treat an asthmatic attack. In an *in vivo* situation, the response might be much more complicated than that in an *in vitro* situation.

In conclusion, vardenafil could reduce methacholine-induced contraction of the tracheal smooth muscle. High concentrations of vardenafil might actually inhibit parasympathetic function of the trachea. Vardenafil might reduce asthmatic attacks when administered to ED patients.

Acknowledgments

This work was supported in part by the Taipei Medical University (104TMU-SHH-17) and the Ministry of Science and Technology, R.O.C. (MOST 104−2314-B038-58).

References

1. Lin CS, Lin G, Xin ZC, Lue TF. Expression, distribution and regulation of phosphodiesterase 5. *Curr Pharmaceut Des* 2006;**12**:3439−57.
2. Mostafa T. Useful implications of low-dose long-term use of PDE-5 inhibitors. *Sleep Med Rev* 2016;**4**:270−84.
3. Minareci E, Sadan G. An evaluation of vardenafil as a calcium channel blocker in pulmonary artery in rats. *Indian J Pharmacol* 2014;**46**:185−90.
4. Li Z, Lu H. New application of phosphodiesterase-5 inhibitors in uroandrology. *Zhonghua Nan Ke Xue* 2013;**19**:675−7.
5. Fang L, Radovits T, Szabo G, Mózes MM, Rosivall L, Kökény G. Selective phosphodiesterase-5 (PDE-5) inhibitor vardenafil ameliorates renal damage in type 1 diabetic rats by restoring cyclic 3', 5' guanosinemonophosphate (cGMP) level in podocytes. *Nephrol Dial Transplant* 2013;**28**:1751−61.
6. Fan Y-F, Zhang R, Jiang X, Wen L, Wu DC, Liu D, et al. The phosphodiesterase-5 inhibitor vardenafil reduces oxidative stress while reversing pulmonary arterial hypertension. *Cardiovasc Res* 2013;**99**:395−403.
7. Ghofrani HA, Reichenberger F, Kohstall MG, Mrosek EH, Seeger T, Olschewski H, et al. Sildenafil increased exercise capacity during hypoxia at low altitudes and at Mount Everest base camp: a randomized, double-blind, placebo-controlled crossover trial. *Ann Intern Med* 2004;**141**:169−77.
8. Prasad S, Wilkinson J, Gatzoulis MA. Sildenafil in primary pulmonary hypertension. *N Engl J Med* 2000;**343**:1342.
9. Aziret M, Irkorucu O, Reyhan E, Erdem H, Das K, Ozkara S, et al. The effects of vardenafil and pentoxifylline administration in an animal model of ischemic colitis. *Clinics* 2014;**69**:763−9.
10. Laties AM, Fraunfelder FT. Ocular safety of Viagra (sildenafil citrate). *Trans Am Ophthal Soc* 1999;**97**:115−25.
11. Chiu F-H, Wang H-W. The evaluation of thioperamide effects using rat's trachea model. *Clin Exp Otorhinolaryngol* 2013;**6**:1−6.
12. Kao C-H, Chu Y-H, Wang H-W. Effects of lidocaine on isolated rat's tracheal smooth muscle. *Eur Arch Oto-Rhino-Laryngol* 2010;**267**:817−20.
13. Rajfer J, Aronson WJ, Bush PA, Dorey FJ, Ignarro LJ. Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. *N Engl J Med* 1992;**326**:90−4.
14. Hallen K, Wiklund NP, Gustafsson LE. Inhibitors of phosphodiesterase 5 (PDE 5) inhibit the nerve-induced release of nitric oxide from the rabbit corpus cavernosum. *Br J Pharmacol* 2007;**150**:353−60.

15. Raeburn D, Advenier C. Isoenzyme-selective cyclic nucleotide phosphodiesterase inhibitors: effects on airways smooth muscle. *Int J Biochem Cell Biol* 1995;**27**:29−37.
16. Bratton DL, Tanaka DT, Grunstein MM. Effects of temperature on cholinergic contractility of rabbit airway smooth muscle. *Am J Phys* 1987;**63**:1933−41.
17. Gonzalez O, Santacana GE. Effect of low temperature on tracheal smooth muscle contractile and relaxing responses evoked by electrical field stimulation. *Puert Rico Health Sci J* 2001;**20**:237−44.
18. Yau KI, Hwang TL. The nonadrenergic noncholinergic system can modulate the effect of prokinetic agents on contractile response of isolated Guinea pig trachea segments to electrical field stimulation. *J Formos Med Assoc* 2002;**101**:695−9.
19. Richalet JP, Gratadour P, Robach P, Pham I, Déchaux M, Joncquiert-Latarjet A, et al. Sildenafil inhibits altitude-induced hypoxemia and pulmonary hypertension. *Am J Respir Crit Care Med* 2005;**171**:275−81.
20. Wang H-W, Jackson RT. Do cholinergic neurons directly innervate nasal blood vessels? *Rhinology* 1988;**26**:139−46.