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European Journal of Pharmacology 584 (2008) 398–404
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Bronchodilatory effects of *S*-isopetasin, an antimuscarinic sesquiterpene of *Petasites formosanus*, on obstructive airway hyperresponsiveness

Ling-Hung Lin [a], Tzu-Jung Huang [b], Sheng-Hao Wang [b], Yun-Lian Lin [c], Sheng-Nan Wu [d], Wun-Chang Ko [b,*]

[a] *Department of Dentistry, Taipei Medical University Hospital, Taipei 110, Taiwan*
[b] *Graduate Institute of Pharmacology, Taipei Medical University, Taipei 110, Taiwan*
[c] *National Research Institute of Chinese Medicine, Taipei 112, Taiwan*
[d] *Department of Physiology, National Cheng Kung University, Tainan 701, Taiwan*

Received 25 October 2007; received in revised form 3 February 2008; accepted 13 February 2008
Available online 19 February 2008

Abstract

In the presence of neostigmine (0.1 μM), *S*-isopetasin competitively antagonized cumulative acetylcholine-induced contractions in guinea pig trachealis, because the slope [1.18±0.15 (n=6)] of Schild's plot did not significantly differ from unity. The pA_2 value of *S*-isopetasin was calculated to be 4.62±0.05 (n=18). The receptor binding assay for muscarinic receptors of cultured human tracheal smooth muscle cells (HTSMCs) was performed using [^{3}H]-*N*-methylscopolamine ([^{3}H]-*N*MS). Saturation binding assays were carried out with [^{3}H]-*N*MS in the presence (non-specific binding) and absence (total binding) of atropine (1 μM). Analysis of the Scatchard plot (y=0.247–1.306x, r^2=0.95) revealed that the muscarinic receptor binding sites in cultured HTSMCs constituted a single population (n_H=1.00). The equilibrium dissociation constant (K_d) and the maximal receptor density (B_{max}) for [^{3}H]-*N*MS binding were 766 pM and 0.189 pmol/mg of protein, respectively. The $-\log IC_{50}$ values of *S*-isopetasin, methoctramine, and 1,1-Dimethyl-4-diphenylacetoxypiperidinium iodide (4-DAMP) for displacing 0.4 nM [^{3}H]-*N*MS-specific binding were 5.05, 6.25, and 8.56, respectively, which suggests that [^{3}H]-*N*MS binding is predominantly on muscarinic M_3 receptors of cultured HTSMCs. The inhibitory effects of *S*-isopetasin on enhanced pause (P_{enh}) value were similar to that of ipratropium bromide, a reference drug. The duration of action of *S*-isopetasin (20 μM), also similar to that of ipratropium bromide (20 μM), was 3 h. In contrast to ipratropium bromide, which non-selectively acts on muscarinic receptors, *S*-isopetasin preferentially acts on muscarinic M_3 receptors. In conclusion, *S*-isopetasin may be beneficial as a bronchodilator in the treatment of chronic obstructive pulmonary disease and asthma exacerbations.

Keywords: *S*-isopetasin; Acetylcholine; Muscarinic receptor; [^{3}H]-*N*-Methylscopolamine; Guinea pig trachealis; Human tracheal smooth muscle cell

1. Introduction

In 1993, Brune et al. (1993) reported that the extract of *Petasites hybridus* L. (Compositae), had been used as a therapeutic spasmolytic agent for gastrointestinal tract spasms and asthmatic attacks in the late Middle Ages in Europe. Recently, Ze 339, an extract of the butterbur plant, was approved by the Swiss government agency Swissmedic as an antiallergic drug (Tesalin; Zeller AG, Romanshorn, Switzerland) to treat seasonal allergic rhinitis. In a study by Schapowal (2002), the clinical effects of Ze 339 were similar to those of cetirizine, an antagonist of histamine receptor subtype 1, although Ze 339 has been reported to have no effect on skin test reactivity induced by different stimuli (Gex-Collet et al., 2006). *Petasites formosanus* Kitamura, a perennial herb and the only indigenous *Petasite*s species in Taiwan, is used as a folk medicine for treating hypertension, tumors, and asthma in Taiwan (Sasaki, 1924). Lin et al. have reported that it contains several new eremophilane-type sesquiterpenes, together with six known compounds, including *S*-petasin, *S*-isopetasin, petasin, and isopetasin (Lin et al., 1998a,b). The content of *S*-petasin in the aerial part of the plant is the most abundant

* Corresponding author. 250 Wu-Hsing St., Taipei 110, Taiwan. Tel.: +886 2 2736 1661x3197; fax: +886 2 2377 7639.
E-mail address: wc_ko@tmu.edu.tw (W.-C. Ko).

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doi:10.1016/j.ejphar.2008.02.034

among these four (Lin et al., 1998b). *S*-petasin ($IC_{50}<10$ μM) was proven to be the most potent in relaxing guinea pig trachea precontracted by histamine, carbachol, KCl, or leukotriene D_4, although *S*-isopetasin ($IC_{50}\cong 10$ μM) has a similar relaxing potency on carbachol and KCl, but almost no effect on histamine and leukotriene D_4 (Ko et al., 2000). We also reported that the relaxant effects of the sulfur-containing petasins, *S*-petasin and *S*-isopetasin, were more potent than those of the non-sulfur-containing petasins, petasin and isopetasin (Ko et al., 2000). The mechanism of the relaxant action of *S*-isopetasin against carbachol in guinea pig trachealis has been reported to be antimuscarinic effect (Ko et al., 2001).

The muscarinic receptors of mammalian airways are classified into M_1–M_5 subtypes. The muscarinic M_1 receptors are distributed throughout the parasympathetic ganglia and exocrine glands and are responsible for cholinergic transmission. The prejunctional muscarinic M_2 autoreceptors are found in the smooth muscle and the myocardium, and they provide negative presynaptic feedback to reduce further release of acetylcholine (Haddad and Rousell, 1998). The muscarinic M_3 receptor subtypes in the airway smooth muscle mediated bronchoconstriction and mucus secretion (Joos, 2000). In rabbit and pig lungs, the occurrence of muscarinic M_4-receptors has been demonstrated (Mak et al., 1993; Chelala et al., 1998). By a combined kinetic and equilibrium labeling technique for radioligand binding assay of muscarinic receptor subtypes and by receptor immunochemistry and immunocytochemistry, a lesser extent of muscarinic M_5 receptors were observed in peripheral blood lymphocytes of asthmatics compared to control individuals (Ricci et al., 2002). When coupled to G proteins, muscarinic M_1, M_3 and M_5 receptors have a stimulatory effect on the target tissue, whereas the muscarinic M_2 and M_4 receptors are inhibitory (Joos, 2000). Currently available inhaled anticholinergic agents, such as ipratropium, oxitropium, and tiotropium bromides, for bronchodilation are non-selective for these subtypes (Restrepo, 2007). The blockade of the muscarinic M_2 receptor by these agents allows further release of presynaptic acetylcholine and may antagonize the bronchodilatory effect of blocking the muscarinic M_3 receptor. The ideal anticholinergic drug for obstructive airway disease should antagonize muscarinic M_3 receptor with little affinity for the muscarinic M_2 receptor (Restrepo, 2007). Therefore, we are interested in investigating *S*-isopetasin, which preferentially acts on tracheal muscarinic M_3, but not cardiac muscarinic M_2 receptors (Ko et al., 2002), as a bronchodilator in obstructive airway hyperresponsiveness. In the present study, the binding properties of *S*-isopetasin on muscarinic receptors in human tracheal smooth muscle cells (HTSMCs) using Scatchard plots (Scatchard, 1949) and the bronchodilator properties of the natural product in murine airway hyperresponsiveness were first time reported.

2. Materials and methods

2.1. Reagents and drugs

S-isopetasin (Fig. 1) was isolated as previously described (Lin et al., 1998a) from the aerial parts of *P. formosanus* Kitamura, and identified by spectral methods, including infrared, mass spectroscopy, 1D- and 2D-nuclear magnetic resonance spectroscopic techniques. The purity of *S*-isopetasin was over 99%. The optical rotation values of *S*-isopetasin was $[\alpha]^{25}_{D}+38.5^{o}$ (c 1.0, $CHCl_3$). acetylcholine, methacholine, indomethacin, neostigmine methyl sulfate, atropine sulfate, ipratropium bromide, ovalbumin, Tris–HCl, dimethyl sulfoxide (DMSO) and methoctramine were purchased from Sigma Chemical (St. Louis, MO, USA). 1,1-Dimethyl-4-diphenylacetoxypiperidinium iodide (4-DAMP) was purchased from Tocris (Avonmouth, UK). [^{3}H]-*N*MS was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Other reagents, such as $CaCl_2$, $MgCl_2$, and NaCl, were of analytical grade. *S*-isopetasin was dissolved in a mixture of DMSO and ethyl alcohol (1: 1). 4-DAMP was dissolved in DMSO, and other drugs were dissolved in distilled water. The final concentration of DMSO or ethyl alcohol was less than 0.1% and did not significantly affect the contractions of the trachealis.

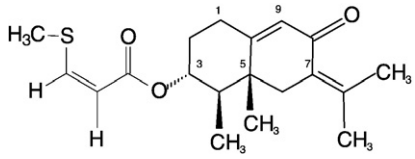

Fig. 1. Chemical structure of *S*-isopetasin (mol. wt., 334).

2.2. Guinea pig trachea

Under a protocol approved by the Animal Care and Use Committee of Taipei Medical University, the following *in vitro* and *in vivo* experiments were performed. Normal male Hartley guinea pigs (National Laboratory Animal Center, Taipei, Taiwan) weighing 500～600 g were sacrificed by cervical dislocation and their tracheas were removed. The isolated guinea pig trachealis were prepared as to our previous report (Ko et al., 2001). To determine the antagonistic effects of *S*-isopetasin and ipratropium bromide against the contractile agonist, acetylcholine was cumulatively added to 5 ml of Krebs solution, containing indomethacin (3 μM) and neostigmine (0.1 μM), and the procedure was repeated until the contractions of the trachealis reached constancy after washout. Then, cumulative concentration–response curves were constructed. The maximal contraction of a trachea without incubation of investigated compound or its vehicle was set as 100%. After the tissues were preincubated with *S*-isopetasin, ipratropium bromide or their vehicles (control) for 15 min, the contractile agonist was also cumulatively added to the Krebs solution. The antagonistic potencies of *S*-isopetasin and ipratropium bromide were expressed as the pA_2 values, when the antagonistic effect on the cumulative concentration–response curve occurred in a competitive manner.

2.3. Culture of human tracheal smooth muscle cells (HTSMCs)

HTSMCs, purchased from Cell Applications (San Diego, CA, USA), were maintained in smooth muscle cell growth

medium (Cell Applications) and equilibrated in a humidified atmosphere of 5% CO_2/95% air at 37 °C. At the beginning of all experiments, unless otherwise states, HTSMCs were plated onto 6-well tissue culture dishes (5×10^5 cells/well). The cells were subcultured weekly after detachment using culture medium containing 1% trypsin. The experiments were performed after the cells had reached confluence (usually 5 ~ 7 days).

2.4. Receptor binding assay

The receptor binding assay for muscarinic receptors was performed using [^{3}H]-*N*MS. Saturation binding assays were carried out in duplicate with [^{3}H]-*N*MS in the presence (non-specific binding) and absence (total binding) of atropine (1 μM). Cultured HTSMCs were washed twice with a Tris–HCl buffer solution (300 mM, pH 7.4) and then incubated for 45 min at 37 °C with [^{3}H]-NMS at various concentrations (62.5 ~ 1000 pM) in a total volume of 0.25 ml of Tris–HCl buffer solution. Competitive binding experiments were performed in the presence of *S*-isopetasin, methoctramine, and 4-DAMP at various concentrations in duplicate with [^{3}H]-*N*MS (0.4 nM). The reaction was terminated by removing the medium and washing the cells three times with a Tris–HCl buffer solution. Cells were solubilized in NaOH (0.1 N); the radioactivity was determined with a liquid scintillation counter (Beckman-Is6500, Fullerton, CA, USA).

2.5. Airway hyperresponsiveness in vivo

Female BALB/c mice at 8 to 12 week of age were obtained from the National Laboratory Animal Center (Taipei, Taiwan). The mice were housed in ordinary cages at 22 ± 1 °C with a humidity of 50 ~ 60% under a constant 12 h/12 h light/dark cycle and provided with ovalbumin-free food and water *ad libitum*. Six mice in each group were sensitized with ovalbumin by an intraperitoneal (i.p.) injection of 20 μg of ovalbumin emulsified in 2.25 mg aluminum hydroxide gel in a total volume of 100 μl on days 0 and 14. A sham (non-sensitized) group of mice received saline instead of ovalbumin was used for comparison. Mice were challenged with 1% ovalbumin in saline for 30 min on days 28, 29, and 30 by ultrasonic nebulization *via* the airway. Two days after the last of the three primary ovalbumin challenges (Kanehiro et al., 2001), airway hyperresponsiveness was assessed in each group. Each group of mice was exposed in the aerosolized *S*-isopetasin (2 ~ 200 μM), ipratropium bromide (0.05 ~ 500 μM), a reference drug, or their vehicle (control) for 5 min. The vehicle was a mixture of alcohol: DMSO: saline (1: 1: 998) and saline alone for *S*-isopetasin and ipratropium bromide, respectively. Fifteen minutes after exposing, the airway hyperresponsiveness was measured in unrestrained animals by barometric plethysmography (Hamelmann et al., 1997) using a whole-body plethysmograph and analyzed using software of Life Science Suite P3 Analysis Modules (Gould, LDS Test and Measurement LLC, Valley View, OH, USA). Mice were placed in the main chamber of the whole-body plethysmograph, and enhanced pause (P_{enh}) values were determined by readings of breathing parameters for 3 min before (baseline) and after nebulization of a phosphate-buffered solution (PBS). Then the P_{enh} values were determined by same method with increasing doses (6.25 ~ 50 mg/ml) of methacholine for 3 min for each nebulization. To study the duration of inhibitory effect on airway hyperresponsiveness of the aerosolized *S*-isopetasin and ipratropium bromide (each 20 μM), each group of six mice was exposed in these drugs and their vehicles for 5 min, and the P_{enh} values were determined by the same method 15 min, 3 h, and 6 h after exposing.

2.6. Data and statistical analysis

The antagonistic effects of *S*-isopetasin on these cumulative concentration–response curves are expressed as pA_2 values, according to the method described by Ariens and van Rosssum (1957): $pA_2 = -\log [B] + \log (DR-1)$, where [B] is the molar concentration of *S*-isopetasin and DR is the dose ratio between concentration of agonist in the presence and absence of *S*-isopetasin. The pA_2 values for *S*-isopetasin were determined using the method of Schild (Schild, 1949; Arunlakshana and

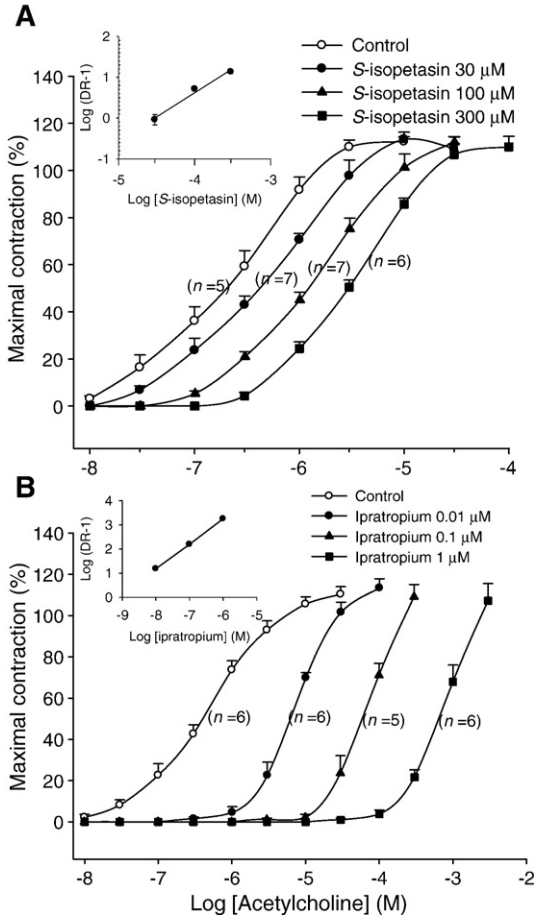

Fig. 2. Inhibitory effects of *S*-isopetasin (30 ~ 300 μM) (A) and ipratropium bromide (0.01 ~ 1 μM) (B) on cumulative acetylcholine-induced contractions in guinea pig trachealis. Each point represents the mean ± S.E.M. The experimental number (*n*) is indicated in figure. The relationship (Schild plot) between the −log (concentration of *S*-isopetasin or ipratropium bromide) and log (DR-1), where DR is the dose ratio, is shown in the inset. The slopes of the Schild plot did not significantly differ from unity.

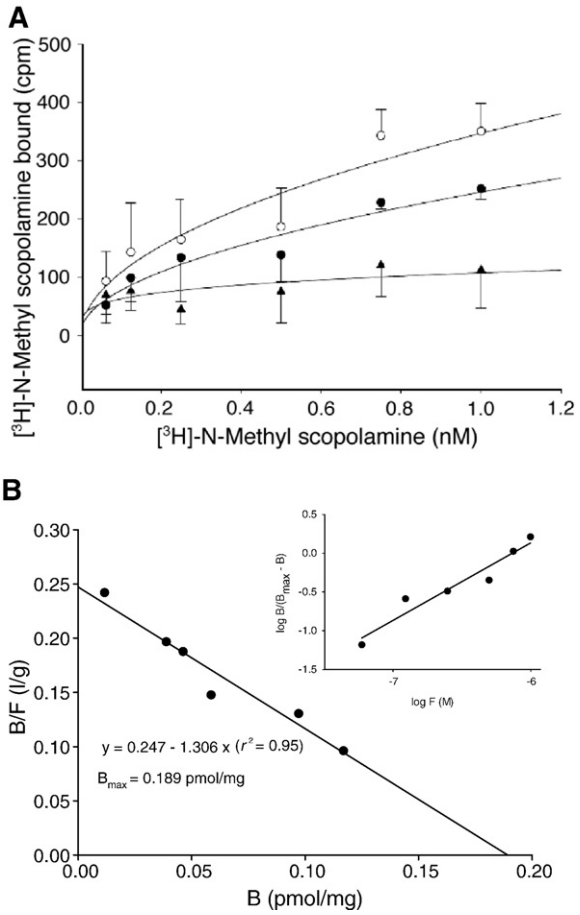

Fig. 3. Saturation binding of [^{3}H]-*N*-methylscopolamine ([^{3}H]-*N*MS) (A) and Scatchard plots (B) of [^{3}H]-*N*MS binding to cultured human tracheal smooth muscle cells. Specific binding (●) is the difference between total (○) and non-specific binding (▲) in the absence and presence of atropine (1 μM), respectively. The inset is a Hill plot of the same data with a coefficient of 1.00. Each point represents the mean±S.E.M. (n=5) in A, and the mean only in B.

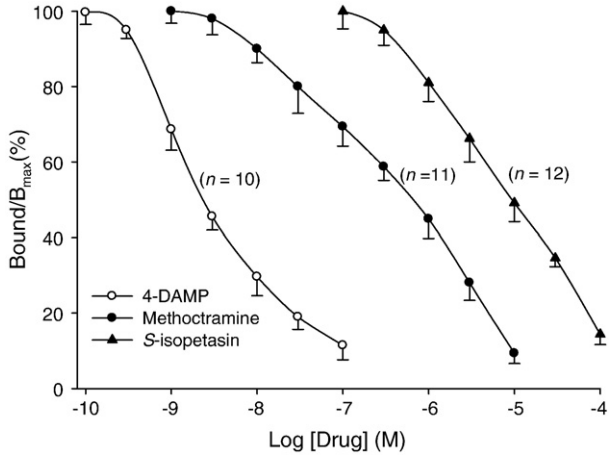

Fig. 4. Log concentration–response curves of *S*-isopetasin, methoctramine (a muscarinic M_2-specific receptor antagonist) and 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide (4-DAMP, a muscarinic M_3-specific receptor antagonist) for displacing [^{3}H]-*N*-methylscopolamine-specific binding in human tracheal smooth muscle cells. Each point represents the mean±S.E.M. The experimental number (n) is indicated in figure.

Schild, 1959). In radioligand binding tests, the maximal number of binding sites (B_{max}) and equilibrium dissociation constant (K_d) were obtained by the Scatchard analysis (Scatchard, 1949) of saturation binding. The Hill plot was derived from the same data of the saturation binding. The $-\log IC_{50}$ value was considered to be equal to the negative logarithm of the molar concentrations of *S*-isopetasin at which half of the B_{max} was displaced. The IC_{50} value was calculated by linear regression. All values are shown as the mean±S.E.M.. Differences among these values were statistically calculated by one-way analysis of variance, then determined by Dunnett's test. The difference between the two values, however, was determined using Student′s unpaired *t*-test. Differences were considered statistically significant for P values<0.05.

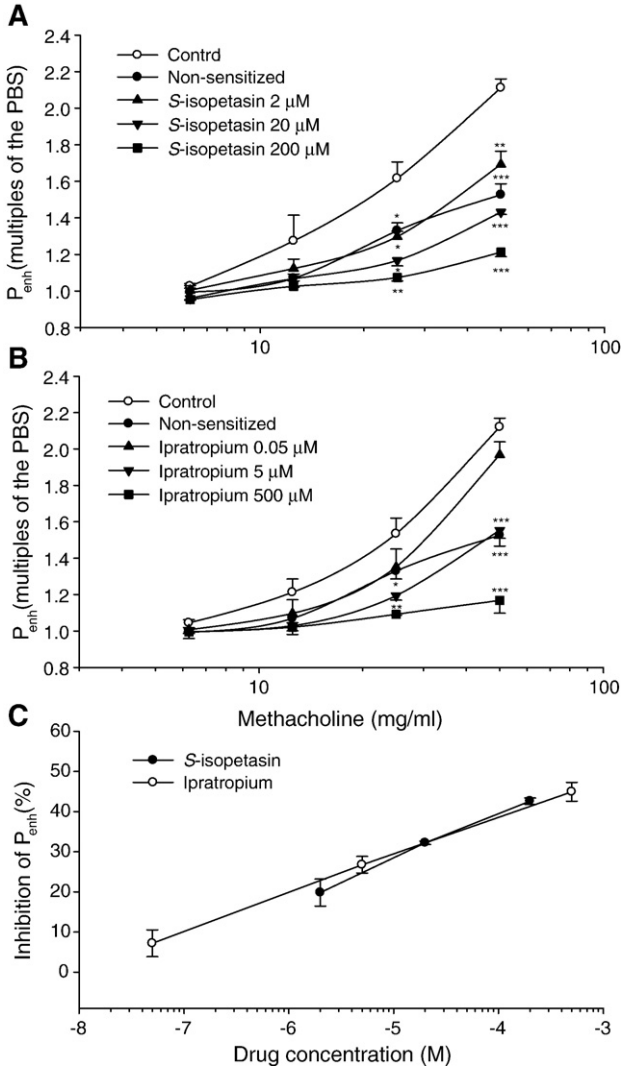

Fig. 5. Effects of aerosolized *S*-isopetasin (2～200 μM) (A) and ipratropium bromide (5～500 μM) (B) on the aerosolized methacholine (6.25～50 mg/ml)-induced enhanced pause (P_{enh}) values in sensitized and challenged mice. The log concentration-inhibition on P_{enh} values of *S*-isopetasin and ipratropium bromide at 50 mg/ml of methacholine nebulization (C). *P<0.05, **P<0.01, *** P<0.001 when compared with the vehicle (control). Each point represents the mean±S.E.M. The number in each group of mice was 6.

3. Results

3.1. Effects of S-isopetasin and ipratropium bromide on acetylcholine-induced contractions in guinea pig trachealis

In the presence of neostigmine (0.1 μM), *S*-isopetasin competitively antagonized cumulative acetylcholine-induced contractions in guinea pig trachealis (Fig. 2A), because the slope [1.04±0.29 (n=6)] of the Schild plot did not significantly differ from unity (Fig. 2A inset). In our experimental conditions, the tension of maximal contraction induced by acetylcholine (10 μM) without antagonist was 1500±30 mg (n=26). Similarly, ipratropium bromide competitively antagonized cumulative acetylcholine-induced contraction in guinea pig trachealis (Fig. 2B), because the slope [1.18±0.15 (n=6)] of the Schild plot did not significantly differ from unity (Fig. 2B inset). The pA_2 values of *S*-isopetasin and ipratropium bromide were calculated to be 4.62±0.05 (n=18) and 9.21±0.03 (n=14), respectively.

3.2. Receptor binding and displacement by S-isopetasin on HTSMCs

For the saturation binding assays of HTSMCs, the non-specific binding was subtracted from the total binding to produce a specific binding curve (Fig. 3A). Analysis of the Scatchard plot (Fig. 3B) revealed that the muscarinic receptor binding sites in cultured HTSMCs constituted a single population (n_H=1.00, Fig. 3B inset). The equilibrium dissociation constant (K_d) and the maximal receptor density (B_{max}) for [^{3}H]-*N*MS binding was 766 pM and 0.189 pmol/mg of protein, respectively. The protein content in each well was 83.95±12.5 μg (n=12). The $-\log IC_{50}$ values of *S*-isopetasin, methoctramine, and 4-DAMP for displacing 0.4 nM [^{3}H]-*N*MS

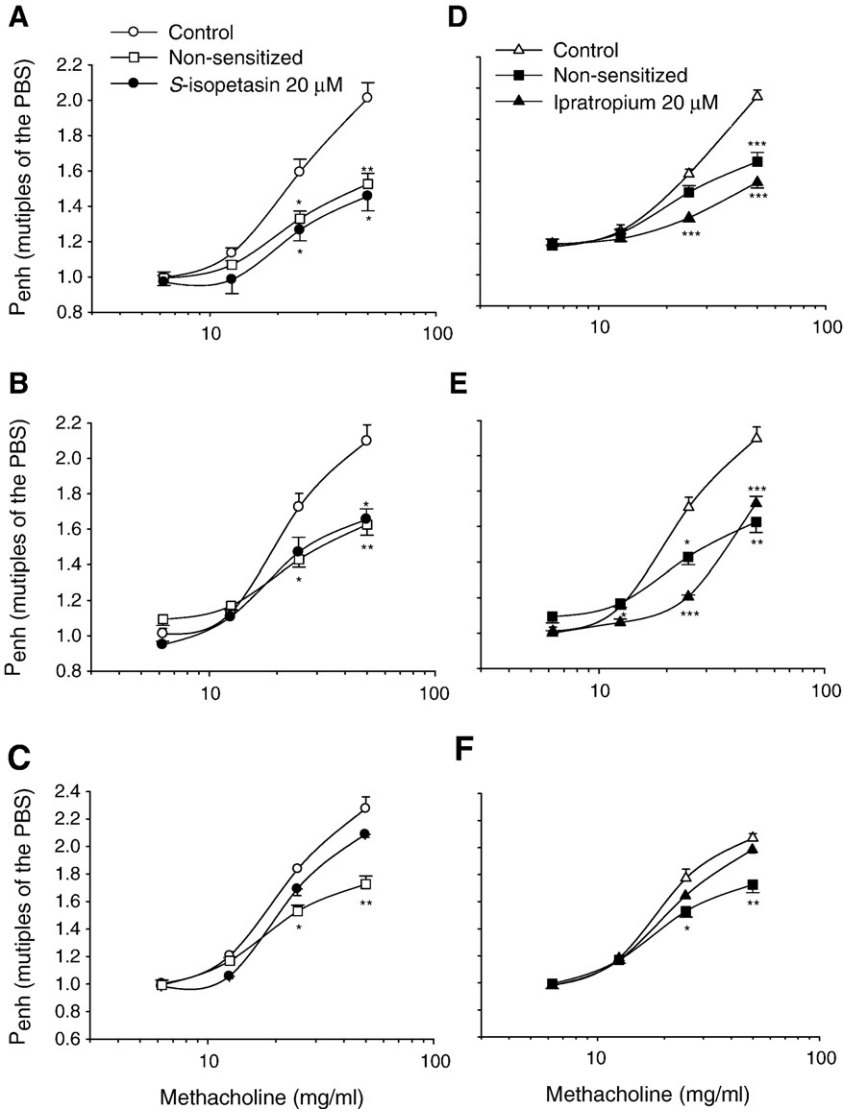

Fig. 6. Duration of aerosolized *S*-isopetasin (20 μM) (A, B, C) and ipratropium bromide (20 μM) (D, E, F) on the aerosolized methacholine (6.25 ~ 50 mg/ml)-induced enhanced pause (P_{enh}) values in sensitized and challenged mice, determined 15 min (A, D), 3 h (B, E) and 6 h (C, F) after exposing of investigated compounds or their vehicles (control). * P<0.05, ** P<0.01, *** P<0.001 when compared with the vehicle (control). Each point represents the mean±S.E.M. The number in each group of mice was 6.

binding were 5.05±0.07, 6.25±0.05, and 8.56±0.04, respectively (Fig. 4).

3.3. Effects and duration of action of S-isopetasin and ipratropium bromide on airway hyperresponsiveness

The P_{enh} values at the baseline for non-sensitized, control (vehicle), and 2, 20, and 200 μM *S*-isopetasin nebulized groups were 4.30±0.53, 4.23±0.26, 3.98±0.34, 4.42±0.23, and 4.15±0.30, respectively, and these values did not significantly differ from each other. The P_{enh} values of PBS nebulization for each group were 4.15±0.36, 4.38±0.25, 4.12±0.16, 4.26±0.34, and 4.05±0.24, respectively, which also did not significantly differ from each other. Administration of nebulized PBS did not affect the P_{enh} value of each baseline group, However, methacholine (6.25–50 mg/ml) concentration-dependently increased P_{enh} values from 1.03±0.01-fold of PBS exposure to 2.11±0.05-fold (Fig. 5A) and from 1.04±0.02-fold of PBS to 2.12±0.05-fold (Fig. 5B) in control sensitized and challenged mice for *S*-isopetasin and ipratropium bromide (a reference drug), respectively. *S*-isopetasin (2–200 μM) nebulization concentration-dependently and significantly inhibited the P_{enh} values at 25 and 50 mg/ml of methacholine exposure (Fig. 5A). Similarly, nebulization of 5–500 μM, but not 0.05 μM, ipratropium bromide significantly inhibited the P_{enh} values at 25 and 50 mg/ml of methacholine exposure (Fig. 5B). The inhibitory effects of *S*-isopetasin nebulization on P_{enh} values at 50 mg/ml of MCh exposure were similar to those of ipratropium bromide (Fig. 5C). The duration of action of 20 μM *S*-isopetasin (Fig. 6A, B) on the P_{enh} values was also similar to that of 20 μM ipratropium bromide (Fig. 6D, E) and significantly lasted 3 h. In contrast to non-sensitized (sham) group, the inhibitory effects of both agents disappeared 6 h after nebulization (Fig. 6C, F).

4. Discussion

In the present results, the inhibitory effect of inhaled *S*-isopetasin (2∼200 μM) on P_{enh} value was similar to that of ipratropium bromide in the same concentration range at 50 mg/ml of methacholine exposure (Fig. 5C). The duration of action of *S*-isopetasin (20 μM), also similar to that of ipratropium bromide (20 μM) was 3 h (Fig. 6), although the concentration (20 μM) chosen from the crossing of both log concentration–response curves of *S*-isopetasin (2∼200 μM) and ipratropium bromide (0.05∼500 μM) may be not enough. Ipratropium bromide is available as a nebulizable solution of 0.02% concentration in a 2.5 ml vial, which is aproximately equal to 500 μM, and requires administration every 6–8 h (Restrepo, 2007). In other words, the duration of action of both drugs may be longer if we use 200 μM or 500 μM instead of 20 μM.

Although the pA_2 value of ipratropium bromide against acetylcholine-induced contractions in guinea pig trachealis was significantly greater than that of *S*-isopetasin in the present results, the inhibitory effects on P_{enh} values or durations of action of both compounds were similar. The reason may be that ipratropium bromide has non-selective on muscarinic receptors, but *S*-isopetasin preferentially acts on muscarinic M_3, but not M_2, receptors (Ko et al., 2002). The blockade of the muscarinic M_2 receptor subtype by ipratropium bromide allows further release of presynaptic acetylcholine in *vivo*, but not in *vitro*, and may antagonize the bronchodilatory effect of blocking the muscarinic M_3 receptors. In guinea pig trachealis, the proportion of muscarinic M_2 receptor population outnumbers the M_3 receptor population by 4: 1 or more. Activation of muscarinic M_3 receptors *via* the G protein, G_q, results in increased polyphosphoinositide hydrolysis and release of Ca^{2+} ions from the sarcoplasmic reticulum, and consequently causes contraction (Hulme et al., 1990). The function of the predominant muscarinic M_2 receptor population, which is characteristically coupled to the guanine nucleotide-binding proteins, G_i or G_k, may be to inhibit β-adrenoceptor-stimulated adenylate cyclase activity and consequently oppose relaxation (Fernandes et al., 1992). The antagonistic effect of *S*-isopetasin against carbachol occurs *via* inhibition of neither total cyclic nucleotide phosphodiesterase (PDE) (Ko et al., 2001) nor PDE isozymes 1∼5 (data not shown).

In cultured HTSMCs, the muscarinic cholinoceptor binding sites constituted a single population (n_H=1.00), according to the analysis of the Scatchard plot. To our knowledge, the K_d and the B_{max} for [^{3}H]-*N*MS binding are for the first time reported in cultured HTSMCs. In the present results, the $-\log IC_{50}$ values of *S*-isopetasin, methoctramine (a muscarinic M_2-selective receptor antagonist), and 4-DAMP (a muscarinic M_3-selective receptor antagonist) for displacing 0.4 nM [^{3}H]-*N*MS binding were 5.05, 6.25, and 8.56, respectively, suggesting that [^{3}H]-*N*MS binding is predominantly on muscarinic M_3 receptors of cultured HTSMCs. This result is supported by a previous report (Mak et al., 1992) which found that muscarinic M_2 cholinoceptors were detected in guinea pig but not in human airway smooth muscles, although muscarinic M_2 receptor-mediated [^{3}H]cyclic adenosine monophosphate formation has been demonstrated in cultured HTSMCs (Widdop et al., 1993). The potency of *S*-isopetasin for replacing [^{3}H]-*N*MS binding in cultured HTSMCs was similar to that against cumulative acetylcholine-induced contractions in guinea pig trachealis, suggesting that *S*-isopetasin may have same effectiveness in human. Therefore, *S*-isopetasin may have benefits as a bronchodilator for treating chronic obstructive pulmonary disease and asthma exacerbations.

Acknowledgment

The support for this work by a grant (95TMU-TMUH-12) from the Taipei Medical University Hospital, Taipei, Taiwan is gratefully acknowledged.

References

Ariens, E.J., van Rosssum, J.M., 1957. pD_x, pA_x and pD'_x values in the analysis of pharmacodynamics. Arch. Int. Pharmacodyn. Ther. 110, 275–299.

Arunlakshana, O., Schild, H.O., 1959. Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14, 48–58.

Brune, K., Bickel, D., Peskar, B.A., 1993. Gastro-protective effects by extracts of *Petasites hybridus*: the role of inhibition of peptido-leukotriene synthesis. Planta Med. 59, 494–496.

Chelala, J.L., Kilani, A., Miller, M.J., Martin, R.J., Ernsberger, P., 1998. Muscarinic receptor binding sites of the M_4 subtype in porcine lung parenchyma. Pharmacol. Toxicol. 83, 200–207.

Fernandes, L.B., Fryer, A.D., Hirshman, C.A., 1992. M_2 muscarinic receptors inhibit isoproterenol-induced relaxation of canine airway smooth muscle. J. Pharmacol. Exp. Ther. 262, 119–126.

Gex-Collet, C., Imhof, L., Brattstrom, A., Pichler, W.J., Helbling, A., 2006. The butterbur extract petasin has no effect on skin test reactivity induced by different stimuli: a randomized, double-blind crossover study using histamine, codeine, methacholine, and aeroallergen solutions. J. Investig. Allergol. Clin. Immunol. 16, 156–161.

Haddad, E.L., Rousell, J., 1998. Regulation of the expression and function of the M_2 muscarinic receptor. Trends Pharmacol. Sci. 19, 322–327.

Hamelmann, E., Schwarze, J., Takeda, K., Oshiba, A., Larsen, G.L., Irvin, C.G., Gelfand, E.W., 1997. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. Am. J. Respir. Crit. Care Med. 156, 766–775.

Hulme, E.C., Birdsall, N.J., Buckley, N.J., 1990. Muscarinic receptor subtypes. Annu. Rev. Pharmacol. Toxicol. 30, 633–673.

Joos, G.F., 2000. Potential usefulness of inhibiting neural mechanisms in asthma. Monaldi. Arch. Chest Dis. 55, 411–414.

Kanehiro, A., Ikemura, T., Makela, M.J., Lahn, M., Joetham, A., Dakhama, A., Gelfand, E.W., 2001. Inhibition of phosphodiesterase 4 attenuates airway hyperresponsiveness and airway inflammation in a model of secondary allergen challenge. Am. J. Respir. Crit. Care Med. 163, 173–184.

Ko, W.C., Lei, C.B., Lin, Y.L., Chen, C.F., 2000. Relaxant effects of petasins in isolated guinea pig trachea and their structure-activity relationships. Planta Med. 66, 650–652.

Ko, W.C., Lei, C.B., Lin, Y.L., Chen, C.F., 2001. Mechanisms of relaxant action of *S*-petasin and *S*-isopetasin, sesquiterpenes of *Petasites formosanus*, in isolated guinea pig trachea. Planta Med. 67, 224–229.

Ko, W.C., Wang, S.H., Chen, M.C., Lin, Y.L., Chen, C.F., 2002. *S*-Isopetasin, a sesquiterpene of *Petasites formosanus*, allosterically antagonized carbachol in isolated guinea pig atria. Planta Med. 68, 652–655.

Lin, Y.L., Mei, C.H., Huang, S.L., Kuo, Y.H., 1998a. Four new sesquiterpenes from *Petasites formosanus*. J. Nat. Prod. 61, 887–890.

Lin, Y.L., Ou, J.C., Chen, C.F., Kuo, Y.H., 1998b. Eremophilanes from *Petasites formosanus*. Chem. Pharm. Bull. 46, 1807–1809.

Mak, J.C., Baraniuk, J.N., Barnes, P.J., 1992. Localization of muscarinic receptor subtype mRNAs in human lung. Am. J. Respir. Cell Mol. Biol. 7, 344–348.

Mak, J.C., Haddad, E.B., Buckley, N.J., Barnes, P.J., 1993. Visualization of muscarinic m4 mRNA and M4 receptor subtype in rabbit lung. Life Sci. 53, 1501–1508.

Restrepo, R.D., 2007. Use of inhaled anticholinergic agents in obstructive airway disease. Respir. Care 52, 833–851.

Ricci, A., Amenta, F., Bronzetti, E., Mannino, F., Mariotta, S., Tayebati, S.K., 2002. Expression of peripheral blood lymphocyte muscarinic cholinergic receptor subtypes in airway hyperresponsiveness. J. Neuroimmunol. 129, 178–185.

Sasaki, S.I., 1924. Taiwan MinkanYakuyo Shokubustsu Shi (A manual of the medicinal plants of Formosa). Kobun Kan, Taipei, Taiwan, p. 9.

Scatchard, G., 1949. The attraction of proteins or small molecules and ions. Ann. N.Y. Acad. Sci. 51, 660–672.

Schapowal, A., 2002. Randomised controlled trial of butterbur and cetirizine for treating seasonal allergic rhinitis. BMJ 324, 144–146.

Schild, H.O., 1949. pAx and competitive drug antagonism. Br. J. Pharmacol. 4, 277–280.

Widdop, S., Daykin, K., Hall, I.P., 1993. Expression of muscarinic M_2 receptors in cultured human airway smooth muscle cells. Am. J. Respir. Cell Mol. Biol. 9, 541–546.