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Bronchodilating effects of bambuterol on bronchoconstriction in guinea pigs

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KEY WORDS bronchodilator agents; terbutaline; histamine; ovalbumin; bronchoconstriction; respiratory function tests; lung compliance; bethanechol; trachea; lung

ABSTRACT

AIM: To study the effects of bambuterol (Bam) on bronchoconstriction in guinea pigs. **METHODS**: Bronchospasm induced by histamine aerosol, lung resistance (R_L) and dynamic lung compliance (C_{dyn}) changes induced by ovalbumin aerosol *in vivo*, isolated resting lung parenchyma strips, and carbamylcholine-induced tracheal constriction *in vitro* in guinea pig were investigated. **RESULTS**: Bam dose-dependently prolonged the time to histamine-induced collapse. ED_{50} values (95 % confidence limits) of Bam intragastric gavage (ig) after 1 h, 4 h, and 24 h were 0.74 (0.60 - 0.91), 0.75 (0.61 - 0.91) and 1.00 (0.77 - 1.30) $mg \cdot kg^{-1}$, respectively. Bam 2 or 10 $mg \cdot kg^{-1}$ ig 2 h before ovalbumin aerosol partly or almost completely inhibited bronchial challenge of ovalbumin-induced change of R_L and C_{dyn}. Bam 0.1 - 1.0 $\mu mol \cdot L^{-1}$ gave a weak relaxation on isolated tracheal strips induced by carbamylcholine and failed to relax the isolated resting lung parenchyma strips in guinea pig. **CONCLUSION**: Bam showed a long-acting bronchodilation by its slow metabolism *in vivo*.

INTRODUCTION

Bambuterol (Bam), a biscarbamate ester prodrug, is hydrolyzed to terbutaline (Ter) primarily by butyrylcholinesterase, and lung tissue is capable of this metabolic pathway. It is also oxidatively metabolized to products which can be hydrolyzed to Ter[1,2]. The efficacy of Bam has been demonstrated to last 24 h after ingestion. Once-daily Bam 10 - 20 mg had an effect/side effect ratio than Ter 3 mg three times daily and 8 mg controlled release salbutamol twice-daily[3-4], so once-daily administration in the evening is recommended[2]. However, no studies have been performed to confirm pharmacodynamics of Bam in animal model in the past decade. The objective of this study was to evaluate pharmacodynamics of Bam on bronchoconstriction in guinea pigs and its duration of action in comparison with Ter.

MATERIALS AND METHODS

Guinea pigs Hartley guinea pigs of either sex weighing (290 g ± *s* 25 g) were used from Experimental Animal Center of Zhejiang Medical University (Grade Ⅱ, Certificate № 22-9601018 conferred by Zhejiang Medical Laboratory Animal Administration Committee).

Drugs Bam hydrochloride (Astra, Belgium); Ter sulfate (Astra Wuxi Pharmaceutical Co. Ltd, China); isoprenaline hydrochloride (Xin-Yi Pharmaceutical Factory, Shanghai); histamine phosphate (Shanghai Institute of Biochemistry, Chinese Academy of Sciences); egg albumin Ⅱ (Sigma, USA); carbamylcholine (Sigma, USA); pentobarbital sodium (Union, Belgium).

Histamine-induced bronchospasm in guinea pig Bronchospasm responses were induced and evaluated by a method based on our previous description[5]. Briefly, a guinea pig was in a bell cover (4 L) into which was fitted the output port of an

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ultrasonic nebulizer (particle size 1 – 5 μm; Model 402, Heli Medical Instrumental Factory, Shanghai). The output of the nebulizer was adjusted to produce a control collapse time of 80 s (60 – 100 s) in the animals. Bam and Ter were dissolved in 0.9 % NaCl (wt/vol), and administered ig, 1 h, 4 h, and 24 h before 0.2 % histamine aerosol. Response to histamine was measured as the time to collapse, up to 6 min. Animals not collapsing within 6 min after histamine aerosol were considered "completely protected".

Ovalbumin-induced changes of R_L and C_{dyn} in the sensitized guinea pig

Sensitizing procedures Guinea pigs were sensitized by a single ip of ovalbumin 10 mg mixed with aluminum hydroxide 100 mg in saline 1.0 mL per animal. These animals were used 25 – 35 d later for aerosol challenge with ovalbumin.

R_L and C_{dyn} measurement The sensitized guinea pigs were anesthetized with pentobarbital (30 $mg \cdot kg^{-1}$) and placed in a whole body plethysmograph for the measurement of R_L and C_{dyn}[6]. The sensitized animals were treated with Bam or saline 2 h before a 1-min exposure to an aerosol of ovalbumin 50 $mg \cdot L^{-1}$ in saline which was generated in ultrasonic nebulizer. After antigen challenge, R_L and C_{dyn} were monitored for 30 min and maximal changes from baseline for each parameter were recorded. The effect of Bam was determined by comparing the ovalbumin-induced changes in R_L and C_{dyn} after drug treatment with the mean of antigen responses alone in the same guinea pig in previous and successive control periods.

Carbamylcholine-induced guinea pig tracheal contraction *in vitro* The tracheal strips were equilibrated in oxygenized Krebs' solution at 37 ℃ with a resting tone 1.5 g for 1 h. The tracheal strip was pretreated with carbamylcholine 5 $\mu mol \cdot L^{-1}$, and the tissues contracted about a 1.2 g force displacement (80 % of maximal response). $-\lg EC_{50}$, maximal effect (E_{max}), and % of response to Ter 1 $\mu mol \cdot L^{-1}$ were determined by analysis of cumulative isometric concentration-response curves to β_2-adrenoceptor agonists (0.1 – 100 $nmol \cdot L^{-1}$) in tracheal strip contracted submaximally with carbamylcholine 5 $\mu mol \cdot L^{-1}$. The relaxant response was measured with a force transducer (JZ100, Xinhang Engine & Electron Equipment Ltd, Henan Province, China).

Relaxation of isolated guinea pig lung parenchyma strips Lung parenchyma strips (20 mm × 2 mm × 15 mm) were prepared from the inferior margin of right lower lobe, and equilibrated for 0.5 h in oxygenated Krebs' solution at 37 ℃ with a resting tone 0.5 g. Maximal relaxant response was induced by terbutaline 20 $\mu mol \cdot L^{-1}$.

Statistical analysis One-way ANOVA was done on a computer software (SigmaStat 1.01 for Windows 95, 1992, Jandel Corp, USA), $\bar{x} \pm s$. EC_{50} (95 % confidence limits) were calculated and compared by weighted probit analysis of Bliss method.

RESULTS

Effect of Bam and Ter on bronchospasm induced by histamine aerosol in guinea pig Saline animals generally collapsed less than 100 s after a 10-s exposure to histamine aerosol. Bam dose-dependently prolonged the time to histamine-induced collapse. ED_{50} value (95 % confidence limits) of ig Bam after 1 h, 4 h, and 24 h were 0.74 (0.60 – 0.92), 0.75 (0.61 – 0.91), 1.00 (0.77 – 1.30) $mg \cdot kg^{-1}$ respectively (Tab 1).

Tab 1. Effect of bambuterol and terbutaline on bronchospasm induced by histamine aerosol. $n = 16$ guinea pigs. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* saline. $^eP < 0.05$ *vs* terbutaline.

Drugs/$mg \cdot kg^{-1}$		Collapse time/s 1 h	4 h	24 h
Saline		92 ± 11	81 ± 10	80 ± 12
Bambuterol	0.5	99 ± 15[a]	95 ± 16[a]	92 ± 11[a]
	1.0	142 ± 35[b]	141 ± 24[b]	116 ± 23[b]
	2.0	185 ± 33[c]	173 ± 23[ce]	150 ± 18[ce]
Terbutaline	2.0	219 ± 31[c]	276 ± 22[c]	99 ± 22[a]

Effects of Bam on R_L and C_{dyn} induced by ovalbumin aerosol in sensitized guinea pig Bronchial challenge of ovalbumin-sensitized guinea pigs induced 203 % increase of R_L and 51 % reduction of C_{dyn} with maximal response at 1 min. Mean value increase of R_L and mean value reduction of C_{dyn} from 1 to 30 min after antigen challenge were up to 114 % ± 60 % and 43 % ± 13 %, respectively in saline group.

Bam 2 and 10 mg · kg^{-1} ig prevented bronchial challenge-induced maximal value increase of R_L by 115 %, 7 % and reduction of C_{dyn} by 35 %, 2 % at 1 min, mean value increase of R_L by 83 % ± 49 %, 9 % ± 9 % and mean value reduction of C_{dyn} by 34 % ± 13 %, or increase 10 % ± 24 % from 1 to 30 min, respectively. Bam 10 mg · kg^{-1} ig before ovalbumin aerosol almost completely inhibited the antigen-induced pulmonary function changes (Fig 1).

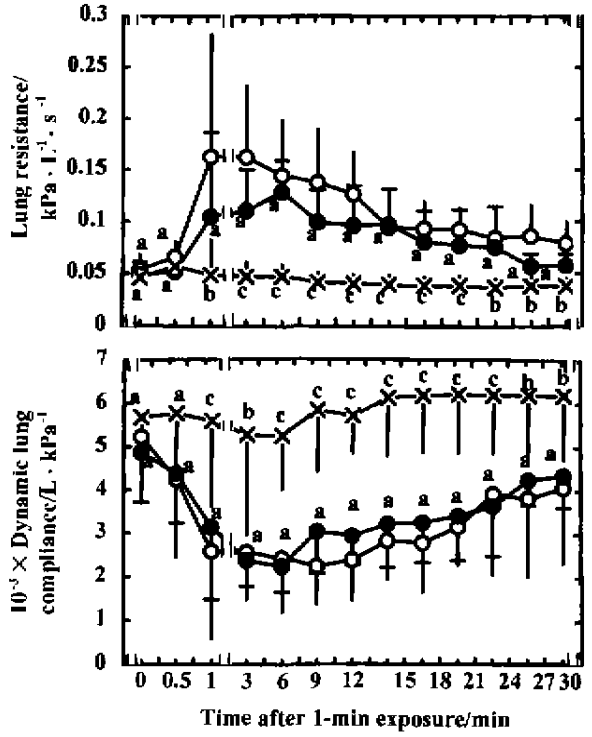

Fig 1. Effect of bambuterol on R_L and C_{dyn} challenged with ovalbumin aerosol in sensitized guinea pig. Saline (○), bambuterol 2 mg · kg^{-1} (●), or 10 mg · kg^{-1} (×), ig 2 h before exposure to ovalbumin aerosol 50 mg · kg^{-1}. n = 10 guinea pigs. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* saline.

Effects of Bam, Ter, and isoprenaline on carbamylcholine-induced tracheal contraction *in vitro* Bam 100 nmol · L^{-1} caused a weak relaxation of carbamylcholine-induced isolated guinea pig tracheal strips, E_{max} = 9 % ± 6 %, while Ter and isoprenaline yielded potent relaxations, EC_{50} (95 % confidence limits) = 1.8 (0.3 – 10.2) and 11 (2.8 – 46) nmol · L^{-1}, E_{max} = 81 % ± 14 % and 69 % ± 20 %, respectively (Fig 2).

Effects of Bam and Ter on the isolated guinea pig lung parenchyma strips Bam 1 μmol · L^{-1} showed no significant relaxation produced on the isolated guinea pig lung parenchyma strips, E_{max} = 8.6 % ± 3.2 %. Ter produced potent relaxation, EC_{50} (95 % confidence limits) = 0.7 (0.03 – 19.7) nmol · L^{-1} and E_{max} = 84 % ± 8 % (Fig 3).

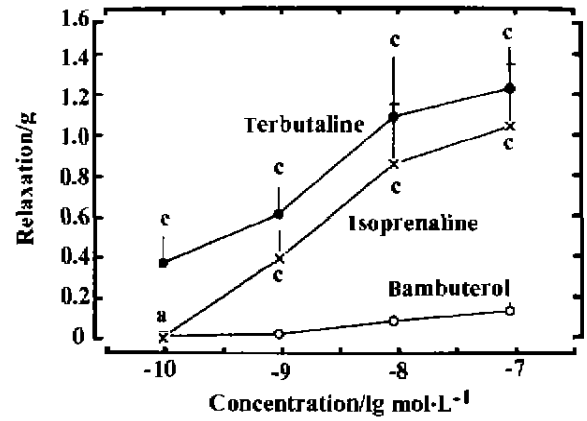

Fig 2. Effects of bambuterol, terbutaline, and isoprenaline on carbamylcholine-induced guinea pig tracheal contraction *in vitro*. n = 8 guinea pigs. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* bambuterol.

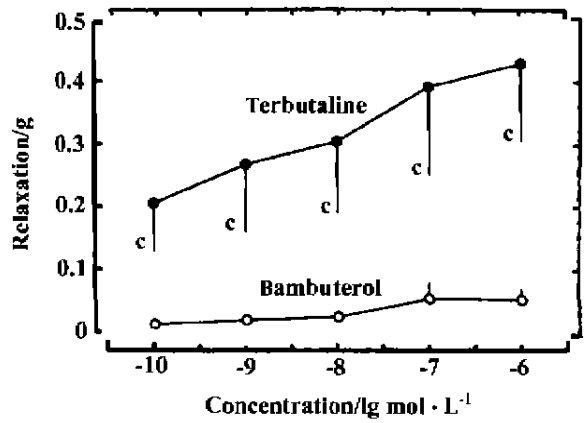

Fig 3. Effects of bambuterol and terbutaline on isolated guinea pig lung parenchyma strips. n = 8 guinea pigs. $\bar{x} \pm s$. $^cP < 0.01$ *vs* bambuterol.

DISCUSSION

The results of this study demonstrated that Bam exerted a protective effect with a longer-acting and an equivalent potency in histamine-induced bronchospasm in comparison with Ter in the same dosage. The result was similar to clinical pharmacodynamics[4]. Ovalbumin mixed with aluminum hydroxide as an antigen

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had been confirmed to produce IgE antibody after injection in guinea pig. R_L and C_{dyn} as parameters respectively reflected large airway and small airway of pulmonary function. Bam 10 mg · kg^{-1} almost completely inhibited ovalbumin-induced changes of R_L and C_{dyn}, suggesting that it had a bronchodilating effect in the sensitized guinea pigs. Bam had a weakly relaxant effect on airway smooth muscle *in vitro*, suggesting that its action was related to its metabolism characteristics. Bam is not only metabolized to Ter by hydrolysis, but also an oxidative metabolism that takes place at the carbonate methyl groups in liver[7]. Bam failed to relax guinea pig lung parenchyma strips in the experiment through a distribution study which was performed in mice with a single dose of tritium-labeled Bam, *in vivo*. Bam and its metabolites, including Ter, were found in higher proportions in the lungs than in other tissues compared with plasma levels[8].

In conclusion, Bam was a long-acting bronchodilator in guinea pigs by its slow metabolism *in vivo*. Bam may offer a significant advantage in the treatment of nocturnal asthma and related respiratory disorders in man.

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班布特罗对豚鼠支气管收缩反应的扩张作用

R974.3
R965.2

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关键词 支气管扩张剂；特布他林；组胺；卵白蛋白；支气管收缩；呼吸功能试验；肺顺应性；氨甲酰胆碱；气管；肺

目的：观察班布特罗对豚鼠支气管收缩反应的影响。**方法**：用哮喘发作潜伏期、肺机械功能及离体气管平滑肌松弛试验观察班布特罗的支气管扩张作用。**结果**：班布特罗 ig 后 1 h、4 h 和 24 h 均能延长组胺诱导的豚鼠哮喘潜伏期，ED_{50}（95 % 可信限）分别为 0.74（0.60 – 0.92），0.75（0.61 – 0.91），1.00（0.77 – 1.30）mg·kg^{-1}，其作用持续时间明显长于特布他林。班布特罗 2 或 10 mg·kg^{-1}在抗原攻击前 2 h ig 部分或几乎完全抑制致敏豚鼠抗原攻击引起的气道阻力增加和肺顺应性降低。但在离体试验中，班布特罗对氨甲酰胆碱引起的气管平滑肌收缩反应和静息肺组织条无明显松弛作用。**结论**：班布特罗通过缓慢代谢发挥其长效支气管扩张作用。

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