Abstract. Background: Beta-2 agonists are potent inhibitors of mast cell degranulation in vitro. Intradermally injected they also inhibit mast cell activation in human skin in vivo. To what extent orally administered \( \beta_2 \)-agonists inhibit mast cell degranulation and allergic skin responses in vivo in daily recommended doses remains unclear.

Purpose: The main purpose was to study the effects of oral administered terbutaline and bambuterol on allergen- and codeine-induced histamine release and skin responses in intact human skin in vivo. In addition, control studies were carried out with intradermally injected terbutaline.

Methods: Ten allergic subjects were randomized to receive bambuterol (10 mg tablets twice daily), terbutaline (7.5 mg controlled release tablets twice daily) and corresponding placebo for 5 days with a washout phase of 3 days between treatments in a double-blind, double-dummy, cross-over trial. The patients were studied at the fifth day of each regimen, i.e. at day 5, 13, and 21. Allergen- and codeine-induced histamine release was measured by microdialysis technique. Wheal and flare reactions to allergen, codeine, and histamine were measured planimetrically. Measurements were performed in the morning on day 5 on each regimen before medication and for additional 5 h after administration of the morning dose. In a separate series of experiments in another 10 allergic patients, 1–1,000 nM (0.05–50 pmoles) of terbutaline was injected intradermally for measurement of histamine release, prostaglandin D2 (PGD2) synthesis and skin responses.

Results: Neither orally administered terbutaline nor bambuterol significantly reduced allergen- or codeine-induced histamine release. Flare reactions to allergen, codeine, and histamine remained unaffected which was also the case for the majority of the wheal reactions. In comparison, intradermally injected terbutaline significantly reduced allergen-induced histamine release, PGD2 synthesis, and skin reactions. Codeine-induced histamine release remained unaffected. Terbutaline significantly reduced flare reactions to codeine and histamine with no effect on wheal reactions.

Conclusions: Terbutaline, in micromolar concentrations, was a potent inhibitor of immediate allergic skin reactions primarily due to inhibition of mast cell degranulation. However orally administered terbutaline, as the active drug itself or released from its pro-drug bambuterol, did not inhibit mast cell activation or allergic skin responses.

Key words: Beta-adrenergic agonists – Histamine – Immediate-type hypersensitivity – Skin tests – Randomized controlled trial

Introduction

Beta-2 agonists like salbutamol and terbutaline are frequently used in the treatment of asthma due to their well-established capacity to relieve bronchoconstriction [1]. Besides their bronchodilator effect several anti-inflammatory effects have been described, e.g. inhibition of anti-IgE induced histamine release and synthesis of prostaglandin D2 and leukotriene C4 in dispersed human lung mast cells in vitro [2]. In vivo, administration of short-acting \( \beta_2 \)-agonists by inhalation and by mouth have shown conflicting results on release of mast cell mediators into the systemic circulation [3, 4].

In contrast to the lung, the human skin is readily available for extensive experimental studies, and it has been used successfully as a model for studies of allergic reactions [5, 6]. Salbutamol and terbutaline inhibit histamine release from human skin mast cells in vitro [2], and in vivo as measured in skin chambers models [7, 8]. Intradermally injected they inhibit immediate wheal and flare reactions to allergens in vivo [7, 9, 10]. There are conflicting data on the effect of oral administered terbutaline on allergic skin responses [11, 12].

Microdialysis technique is a method which allows continuous assessment of mediator release in intact human skin in vivo as well as simultaneous evaluation of skin responses [13]. Recently it was shown by microdialysis technique that salbutamol and salmeterol inhibited mediator release and skin responses in intact human skin [14].
The purpose of the present study was to investigate to what extent terbutaline and bambuterol, a long-acting pro-drug of terbutaline, inhibit immediate-type allergic skin responses following oral administration of the drugs in daily recommended doses. In addition to oral administration, the effects of intradermally terbutaline were also investigated.

Materials and methods

Subjects

A total of 20 subjects (5 females and 15 males aged 22–37 years) participated in two separate experimental protocols. All subjects were allergic to grass pollen based upon symptoms of rhinoconjunctivitis during the pollen season, and the presence of positive skin prick test and allergen specific antibody response (RAST class >2, Pharmacia CAP-system). None had taken any medication within the last month before the study which was conducted outside the grass pollen season. All subjects gave informed consent, and the study was approved by the local ethics committee and the Danish Board of Health.

Drugs

All drugs were supplied from Astra, Sweden. Terbutaline (Bricanyl®), 0.5 mg/ml for intradermally injection was diluted with isotonic saline to working concentrations of 1–1,000 nM. The following drugs were used for oral administration: Terbutaline (Bricanyl®) controlled release tablets, 7.5 mg, and bambuterol (Bambec® tablets, 10 mg), and corresponding placebos. The medication was administered twice daily in a double-dummy, balanced, cross-over design for 5 days with a washout phase of 3 days between treatments, resulting in a 21-days treatment protocol. Randomization was based on computer-generated random numbers. The washout period of 3 days between the treatments was considered appropriate given the half-life of less than 1 day for bambuterol and its active metabolites.

Microdialysis technique

Microdialysis fibers of 216 μm diameters, 2 kDalton molecular weight cut off and a length of 20 mm available for diffusion were made from a hemodialysator. Dialysis fibers were introduced into the skin via 23 gauge guide cannulae as previously described [15]. A local anesthetic cream (EMLA, Astra AB, Södertälje, Sweden) under occlusion (Tegaderm, 3M Health Care Ltd., Loughborough, Leicestershire, U.K.) was applied for 45 min and removed before insertion of fibers. The medication was administered twice daily in a double-dummy, balanced, cross-over design for 5 days with a washout phase of 3 days between treatments, resulting in a 21-days treatment protocol. Randomization was based on computer-generated random numbers. The washout period of 3 days between the treatments was considered appropriate given the half-life of less than 1 day for bambuterol and its active metabolites.

Skin challenge and measurement of skin responses

The following compounds were used for skin challenge: Grass pollen extract (Aquagen, ALK, Hoersholm, Denmark) was diluted in a phenol-buffered diluent (ALK Diluent) to 10 SQ-U/ml. Codeine phosphate was purchased from the institutional pharmacy and diluted with KRB to 0.3 mg/ml. Histamine hydrochloride (SAD, Copenhagen, Denmark) was diluted with KRB to 0.1 mg/ml. All injections were performed with a 0.5 ml syringe with a 27 gauge needle. Skin challenge above micro-dialysis fibers were performed as previously described [14–15]. Injections were made at the midpoint at the 20 mm length in the skin, 1 mm from the center of the fiber.

Immediate wheal and flare reactions to allergen and codeine were outlined by an ink pen after 20 min, the reactions to histamine being measured at 15 min, and being transferred to the subject’s file. The 20 min period for assessment of allergen- and codeine-induced skin reactions were chosen for logistic reasons (sampling of dialysate). Wheal and flare areas were calculated by a scanning device [16].

Effects of intradermally injected terbutaline and bambuterol

This protocol included 10 allergic subjects (10 males aged 23–37 years). Four microdialysis fibers were inserted in each forearm 2 h before any skin testing. Fibers in one arm were challenged with allergen, whereas the fibers in the other arm were challenged with codeine. It was randomly determined (computer-generated random numbers) which fibers were to be used at various time points. The subjects received four days treatment with terbutaline, bambuterol or placebo, and they were examined on the morning of the fifth day of each treatment regime. The first examination, time 0, was performed before administration of the morning dose for that particular treatment. The purpose was to test for duration of effect from the previous 4 days of dosing. Histamine release and skin reactions induced by intradermally injected allergen (10 SQ-U/ml, 25 μl) and codeine (0.3 mg/ml, 25 μl) were examined at skin sites above dialysis fibers and skin reactivity to histamine was assessed in the left upper arm (0.1 mg/ml, 25 μl). Then, the subjects received their morning dose of the study drug and examinations were repeated 1, 2 and 5 h after. This sequence of experiments were performed at Day 5, 13, and 21 on terbutaline (with bambuterol placebo), bambuterol (with terbutaline placebo) or placebo (both terbutaline and bambuterol placebos) in a double-blind, randomized fashion. A Latin square method was used for determination of the drug sequence.

To investigate any relationship between drug effects and drug levels, the concentrations of terbutaline and bambuterol in venous blood and skin dialysate were analyzed. Two dialysis fibers were inserted in the right upper arm at each investigation. Dialysate was collected every 30 min before and up to 5 h after administration of study drug on day 5, 13, and 21. Dialysates from two fibers were pooled to increase sampling volume. No attempts were made to calibrate the dialysis probe in vivo in order to measure absolute extracellular skin concentrations of the drugs. Dialysate samples were immediately stores at –20°C until analysis. Venous blood samples of 10 ml were drawn from an indwelling catheter in a cubital vein into sodium-heparinized sampling tubes containing an esterase inhibitor (the bambuterol analogue D2456, 1 mM [0.1 micro-moles]). The samples were immediately centrifuged at 1400 g and plasma was transferred into polystyrene tubes and stored at –20°C until analysis. Blood samples were taken hourly from time 0 to 5 h.