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Research Article

Anti-Bronchoconstrictor Activities and Side Effect Potential of Inhaled Formoterol and (*S,R*)-Epiformoterol in the Rhesus Monkey

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Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT In a recent patent, the (*S,R*) isomer of epiformoterol was claimed to be a bronchodilator with reduced adverse effects compared to racemic formoterol. We initiated the present study to seek direct evidence for the claim. Anaesthetised, spontaneously breathing rhesus monkeys were set up for measuring airways resistance. Blood pressure and heart rate were measured concomitantly to gauge systemic exposure and the potential for side effects. Formoterol, 1.2 μg/kg, administered by aerosol, induced rapidly developing, sustained inhibition of the bronchoconstrictor responses to aerosolised methacholine accompanied by tachycardia. (*S,R*)-epiformoterol, 63 μg/kg, induced anti-bronconstrictor effects and an associated tachycardia, which were superimposable with those induced by racemic formoterol at 1.2 μg/kg. Thus, (*S,R*)-epiformoterol is a long-acting anti-bronchoconstrictor agent in the rhesus monkey and causes an associated tachycardia. This profile is not qualitatively different from that of racemic formoterol but does differ from that of salmeterol, which, in this model, shows lower efficacy and more pronounced tachycardia for an equivalent degree of anti-bronchoconstrictor activity. Thus, the claim that (*S,R*)-epiformoterol shows bronchodilator activity with reduced adverse effect potential compared to racemic formoterol is not supported by the present data. Drug Dev. Res. 57:1–5, 2002. © 2002 Wiley-Liss, Inc.

Key words: bronchodilation; β_2 adrenoceptor agonists; adverse effects; tachycardia

INTRODUCTION

Formoterol, whose chemical name is (±)-*N*-(2-Hydroxy-5-{1-hydroxy-2-[-2-(4-methoxy-phenyl)-1-methyl-ethylamino]-ethyl}-phenyl)-formamide, is a long-acting β_2 adrenoceptor agonist that is used as a bronchodilator agent in asthma [Bartow and Brogden, 1998; Beasley et al., 2000; Moore et al., 1998; Redington and Rees, 1998]. The structural constitution corresponding to formoterol contains two dissimilar chiral centres, which results in two possible diastereoisomeric forms; thus, four enantiomeric configurations are possible: formoterol comprising the (*R,R*) and (*S,S*) enantiomeric forms of the *like*-diastereoisomer, and epiformoterol comprising the (*R,S*), and (*S,R*) enantiomeric forms of the *unlike*-diastereoisomer. The marketed form of formoterol is a racemic mixture of the two *like* enantiomers; the β_2 agonist activity resides in the (*R,R*)-enantiomer whilst the (*S,S*) form is essentially inactive [Fozard and Buescher, 2001; Schmidt et al., 2000; Trofast et al., 1991].

In a recent patent, the (*S,R*) isomer of epiformoterol was claimed to be a bronchodilator with reduced adverse effects compared to racemic formoterol [Jerussi and Senanayake, 2000]. Since the case is largely hypothetical (no experimental data in support of

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the claim is included), we initiated the present study to seek direct evidence for the claim. Thus, we have defined the anti-bronchoconstrictor activity of inhaled (*S,R*)-epiformoterol in a model of methacholine-induced bronchoconstriction in the rhesus monkey [Fozard and Buescher, 2001] and compared its potency and duration of action with racemic formoterol. Blood pressure and heart rate were measured concomitantly to gauge systemic exposure and the potential for side effects.

MATERIALS AND METHODS

Drugs and Solutions

Racemic formoterol was synthesised by Novartis Pharma Basel, Switzerland and (*S,R*)-epiformoterol (enantiomeric purity >99.9%) was synthesised at Novartis, Horsham Research Centre, Horsham, UK. The fumarate salts were used in the study and doses refer to the salt forms. Ketamine (Narketan) was supplied by Chassot; thiopentone (Pentothal) was from Abbott; xylocaine spray, 10%, was from Astra; methacholine chloride was from Research Organics, Cleveland, OH. Methacholine, racemic formoterol, and (*S,R*)-epiformoterol were dissolved in saline. Solutions for nebulisation were prepared freshly prior to each experiment.

Animals

A group of 17 male rhesus monkeys supplied by the Centre for Primatology at the University of Strasbourg, Strasbourg, France, and weighing 6.0–14.6 kg was used. The animals were kept in colonies in a specialised primate unit. The interval between experimental days was a minimum of 3 weeks. All experiments were carried out with the approval of the Veterinary Authority of the City of Basel (Kantonales Veterinaeramt, Basel-Stadt).

General Experimental Procedure

The monkeys were fasted approximately 18 h prior to the experiment. On the day of the experiment, animals were immobilised with ketamine (10 mg/kg i.m.) and anaesthetised with thiopentone (8–11 mg/kg/h), which was infused via a saphenous vein and maintained throughout the experiment. Animals were placed in a left lateral position on a heated operating table and allowed to breathe spontaneously. The larynx, epiglottis, and pharynx were anaesthetised by topical application of xylocaine spray (10% solution; 10–20 mg in total), allowing introduction and placement of a cuffed paediatric endotracheal tube (diameter 4.5 mm).

The following parameters were monitored by a Siemens Sirecust model 1281 (Siemens, Erlangen, D): end tidal CO_2 in the blood (measured at the beginning of the experiment using an infrared CO_2 transducer), body temperature (measured continuously using a thermistor probe inserted into the esophagus), and systolic and diastolic arterial blood pressure (measured using a paediatric cuff). Heart rate and arterial oxygen saturation were measured continuously by pulse oximetry (Pulse Oximeter, Nellcor, Hayward, CA).

Lung function during spontaneous breathing was monitored with a paediatric pulmonary function unit (model 2600, Sensormedics, Yorba Linda, CA) controlled by an IBM 365 PC. The method is based on single breath analysis of passive respiratory mechanics using a pneumotachograph (Hans Rudolph Inc, Kansas City, MO; series 4500A in line with an appropriate breathing interruptor) attached to the endotracheal tube. The system allows measurement of expiratory flow-volume loops after airway occlusion at the end of a tidal inspiration. Airways resistance is calculated from the ratio of airways occlusion pressure to expiratory flow. Measurements of airways resistance (usually 10 readings during a time period of approximately 2 min) were performed immediately after termination of drug administration and at intervals up to 285 min thereafter.

Drug Administration

Racemic formoterol and (*S,R*)-epiformoterol were delivered as an aerosol by tidal breathing using a Cirrhus nebulizer (DHD Medical Products, Canastota, USA). The nebulizer was connected to the endotracheal tube by a T-adaptor and operated at 4 l/min flow of compressed air. The aerosol generated has a median droplet diameter of 3.5 μm. The administered dose [mg/kg] was calculated using the formula:

$$0.3 \times \text{minute volume [l/min]} \times \text{treatment time [min]} \times \text{concentration of compound in aerosol [mg/l]}$$
$$\text{divided by body weight [kg].}$$

The factor 0.3 refers to an assumed 30% retention of the inhaled material in the lung. Because the dose is calculated it varies slightly between individual animals. The doses presented are, therefore, the group mean values. The aerosol concentration was calculated from the total amount of drug aerosolised by the nebulisation at 4 l/min of compressed air.

Experimental Design

Experiments were performed according to the following schedule:
Time 0: Determination of resting cardiovascular and respiratory parameters and airways resistance (baseline 1). Time 15 min: Determination of effect of saline

aerosol administration (vehicle for methacholine; 2-min exposure) on cardiovascular and respiratory parameters and airway resistance. Time 30 min: Determination of initial bronchoconstrictor response (50–90% over baseline) to a submaximal dose of methacholine (predefined for each animal, usually 1.2 mg/ml, occasionally 0.3, 0.6, or 2.5 mg/ml; 2-min exposure). Time 60 min: Determination of cardiovascular and respiratory parameters and airway resistance following recovery from methacholine (baseline 2). Start of aerosol administration of drug or vehicle (10-min exposure). Time 72 min: Determination of effect of drug or vehicle per se on cardiovascular and respiratory parameters and airway resistance. Time 77 min onward: Determination of responses to methacholine at 5, 95, 155, 215, and 275 min after the end of drug or vehicle administration. Baseline parameters were determined 5 min prior to each challenge.

Data Analysis

Data processing was done using the following calculations:

(1) Initial response to methacholine was quantified as the % change from baseline 1.
(2) Effects of drug or vehicle at the end of aerosol treatment were calculated as the % change from baseline 2.
(3) Responses to methacholine following vehicle or drug were quantified as the % change from the baselines measured 5 min before challenge at each time point from 5 min after the end of drug or vehicle administration.
(4) Changes in the response to methacholine at different times following drug or vehicle were calculated as the % change in the initial response to methacholine.

Statistical evaluation was carried out on data expressed as % change of each treatment compared to respective time points of the control group using Student's *t*-test for non-paired observations of the Excel software package (Microsoft). Significance was assumed at the 5% probability level.

RESULTS

The results with racemic formoterol are taken from our earlier study [Fozard and Buescher, 2001]. At a concentration of 0.04 mg/ml in the nebuliser solution (calculated mean dose 1.2 μg/kg), racemic formoterol induced a marked anti-bronchoconstrictor effect that was rapid in onset and submaximal with respect to duration of action over the 275-min observation period [Fozard and Buescher, 2001] (Fig. 1A). The anti-bronchoconstrictor effect was associated with tachycardia (maximum 27% increase in heart rate at the end of the drug administration period), which declined slowly over the observation period (Fig. 1B).

Based on the reported 50-fold lower potency of (*S,R*)-epiformoterol compared to formoterol as a relaxant of the guinea-pig isolated trachea [Jerussi and Senanayake, 2000], a concentration of 2 mg/ml of (*S,R*)-epiformoterol in the nebuliser solution, resulting in a mean dose of 63 μg/kg, was tested. The results obtained with (*S,R*)-epiformoterol at 63 μg/kg were qualitatively and quantitatively similar to those obtained with racemic formoterol at 1.2 μg/kg (Fig. 1). Thus, a marked anti-bronchoconstrictor activity of rapid onset was seen, which declined to control values over the course of the experiment (Fig. 1A). The anti-bronchoconstrictor activity was accompanied by tachycardia (Fig. 1B). Neither racemic formoterol nor (*S,R*)-epiformoterol induced any change in blood pressure (data not illustrated).

DISCUSSION

In an earlier study [Fozard and Buescher, 2001], we showed that the present experimental model can be used to identify several of the key clinical features of the two marketed long-acting β_2 agonists, formoterol and salmeterol; thus effects to suppress bronchoconstrictor responses to methacholine predict their relative potency, efficacy, and duration of action as bronchodilator agents and tachycardia, an index of systemic exposure, their potential for adverse effects. It was of interest, therefore, to use this model to evaluate the recent claim that (*S,R*)-epiformoterol possesses "similar β_2 selectivity to that of the corresponding (*R,R*)-isomer and therefore avoids certain adverse effects associated with the racemic form" [Jerussi and Senanayake, 2000].

In the event, the present data show (*S,R*)-epiformoterol, used at a dose chosen based on its potency relative to racemic formoterol as a relaxant of the guinea-pig isolated trachea [Jerussi and Senanayake, 2000], to have a profile indistinguishable from racemic formoterol with respect to both the anti-bronchoconstrictor effects and associated tachycardia. The similar degree of tachycardia for equivalent bronchodilator activity is of particular significance. The principal adverse effects of β_2 agonists are tremor and cardiovascular effects such as palpitations and tachycardia are the consequence of systemic exposure to the compounds. Clearly, there are no grounds from our data to distinguish between (*S,R*)-epiformoterol and racemic formoterol in their propensity for inducing adverse effects. It should be emphasised that this observation does not conflict with the data presented by Jerussi and Senanayake [2000]. In vitro estimates of

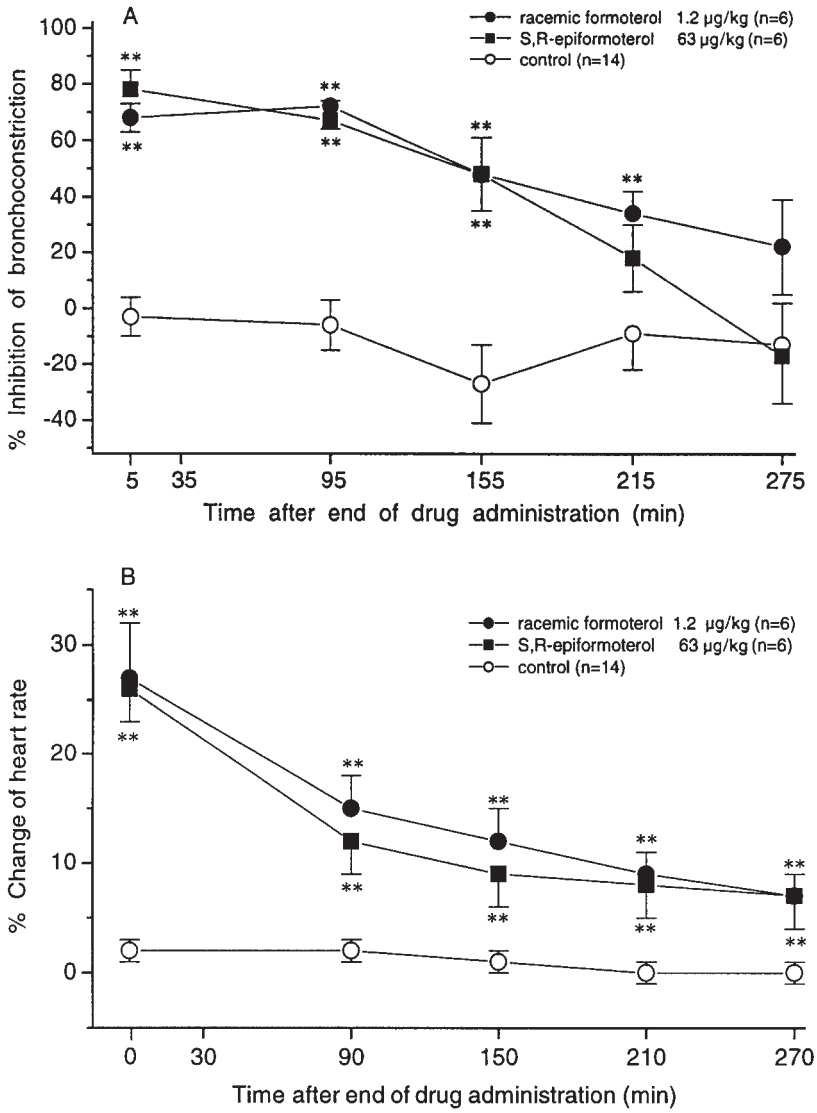

Fig. 1. The effects of (*S,R*)-epiformoterol (63 µg/kg) and racemic formoterol (1.2 µg/kg) on methacholine-induced bronchoconstriction (**A**) and baseline heart rate (**B**) in anaesthetised rhesus monkeys. The compounds were given by aerosol during 10 min at the doses indicated. Control animals received saline. Points represent the mean values from n individual experiments with standard errors. $^{**}P < 0.01$ that the value differs significantly from the respective control value.

relative affinities to β_1 and β_2 receptors, the capacity to stimulate cAMP production, and propensity to cause receptor desensitisation demonstrated no pharmacologically relevant qualitative difference between (*S,R*)-epiformoterol, racemic formoterol or its (*R,R*)-enantiomer.

In conclusion, (*S,R*)-epiformoterol is a long-acting anti-bronchoconstrictor agent in the rhesus monkey and causes an associated tachycardia. This profile is qualitatively similar to that of racemic formoterol and its (*R,R*)-enantiomer [Fozard and Buescher, 2001], although predictably, on the basis of in vitro comparisons of potency, (*S,R*)-epiformoterol is approximately 50 times less potent than racemic formoterol. The profile does, however, differ from that of salmeterol, which, in this model, shows lower efficacy and more pronounced tachycardia for an equivalent degree of anti-bronchoconstrictor activity [Fozard and Buescher, 2001]. Thus, the claim that (*S,R*)-epiformoterol shows bronchodilator activity with reduced adverse effect potential compared to racemic formoterol is not supported by the present data and

may be limited to the entirely hypothetical perception of a formulation/dosing advantage due to the reduced potency of (*S,R*)-epiformoterol relative to racemic formoterol.

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