REVIEW ARTICLE

# Clinical equivalence testing of inhaled bronchodilators

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**KEY WORDS**

β-agonists, bronchodilation, exercise, methacholine, relative potency

**ABSTRACT**

There are no standardized methods to demonstrate in-vivo bioequivalence of inhaled bronchodilators. The most practical method of showing therapeutic equivalence in vivo is by estimating their relative potencies (RP) in clinical efficacy studies, where the RP of bronchodilators may be estimated by comparing either their bronchodilator or bronchoprotective properties. Bronchodilator studies are easier to perform and may better model the physiologic effect of many agents, including inhaled β-agonists. However, it may be difficult to demonstrate steep dose-response for these outcomes, except in cumulative study designs. Bioequivalence trials may be especially challenging when involving pressurized metered-dose inhalers, as a single actuation – the lowest feasible dose to include in the evaluation, may already produce bronchodilation that is at or near the plateau of the dose-response curve. Protection against bronchoconstriction induced by a direct inhaled stimulus like methacholine or histamine affords a reliable and practical method of comparing inhaled bronchodilators and estimating their RP. Inhalational bronchoprovocation testing allows for easier repeatability and quantitation of the stimulus necessary to produce a predetermined degree of bronchoconstriction, and the degree of protection afforded by the bronchoprotection agent. RP studies using adequate methodology are necessary to compare long-acting bronchodilators and both short- and long-acting bronchodilators in patients who are also on inhaled corticosteroids.

**Introduction** There are no standardized methods to demonstrate in-vivo bioequivalence of inhaled bronchodilators. The most practical method of showing therapeutic equivalence in vivo is by estimating their relative potencies (RP) in clinical efficacy studies. $\beta_2$-agonists have two clinically distinct pharmacodynamic effects in asthma: bronchodilation and prevention of bronchoconstriction. The majority of in vivo bioequivalence studies of $\beta_2$-agonists have compared the bronchodilator action of different drugs. Although both responses are mediated through the same pulmonary receptors, they are clinically and physiologically distinct.[1] The nonbronchodilator effects may be studied by comparing the protection against bronchoconstriction caused by direct agents like methacholine and histamine or indirect agents like exercise, cold air, or allergen. The need for these studies becomes particularly relevant with the expiry of patents of innovator inhaled bronchodilator drugs, introduction of inhalers with non-chlorofluorocarbon (CFC) (hydrofluoroalkane – [HFA]) propellants, combination of inhaled bronchodilators with inhaled corticosteroids in single devices and enantiomeric isomers of bronchodilators such as levalbuterol.

This report examines the relative advantages and disadvantages of various outcome measures and study designs.

**Design and methodology issues of bioequivalence studies** The British Association of Lung Research has made some recommendations as to how bronchodilator studies should be conducted.[2] The studies should ideally be performed in asthmatics with a range of disease severity. A range of doses should be compared in a placebo-controlled trial to establish a dose-response curve. The doses that are compared should be on the steep portion of the curve. Patients should be educated in proper inhalation technique and comparisons should be made under conditions when inhalers are primed

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(activated a few times [usually 5] into a spacer before the patient inhales it] and unprimed. The outcome measure, whether it is forced expiratory volume in 1 second ($FEV_1$) (bronchodilation) or $PC_{20}$ (bronchoprotection), should be repeatable. In addition, for bronchoprotection studies, the concentration of methacholine or histamine should be stable during the period of the study. As with any controlled trials, the studies should have enough sample size to make meaningful interpretations. Currently, there are no recommendations as to what constitutes an appropriate sample size. Bioequivalence of clinical efficacy should be reported as RP, calculated preferably by bioassay (Finney method).[3] Three doses of each drug should be compared to make sure that the doses on the steep portion of the dose-response curve are selected for comparison and that the curves are parallel, unless previous studies had clearly identified two doses which are on the steep part of the curve. If the appropriate doses are selected, and if they are not on the plateau of the dose-response curve, the Finney assay gives results comparable to an E-max model. The US Food and Drug Administration considers two inhaled formulations as bioequivalent if the 90% confidence interval (CI) of the RP is between 0.67 and 1.50.[4] The Therapeutics Products Directorate of Health Canada demands tighter CI of RP of between 0.8 and 1.25 to establish bioequivalence.[5]

**Bronchodilator bioequivalence** In order to demonstrate a significant bronchodilator dose-response, subjects ideally should have significant airflow limitation at the start of the study. If there is very little airflow limitation, the dose-response may be shallow. Several studies have reported the bioequivalence and safety of CFC and HFA salbutamol for its bronchodilator effect according to the above recommendations. In a cumulative dose-response study of 24 subjects with mild asthma, Kleerup et al.[6] reported bioequivalence of Proventil-HFA and Ventolin-CFC. The RP of the bronchodilator effect (% change in $FEV_1$) was calculated as 1.08 (90% CI 0.95, 1.23).[7] A post-hoc power calculation using Monte-Carlo simulations of the ratio of the root mean square of the dose effect (s) and slope of the dose-response curve (b) showed that the study had 80% power to estimate RP with a 90% CI of 0.80 to 1.25.[7] Bronchodilator bioequivalence of HFA- and CFC-salbutamol has also been demonstrated in a placebo-controlled, 6-period, crossover study in 26 asthmatics.[8] More recently, bronchodilator bioequivalence of levalbuterol-HFA and racemic salbutamol-HFA was studied in a placebo-controlled, crossover, cumulative study design,[9] using the Finney method, where bioequivalence was demonstrated, with an RP (90% CI) of 1.1 (0.9–1.2).

**Bronchoprotective bioequivalence** **Protection from methacholine-induced bronchoconstriction** In order to perform a bronchoprovocation, the subjects should not have significant airflow limitation at the start of the study. When performed well, inhalation of methacholine produces reproducible bronchoconstriction. Subjects should demonstrate a 4-fold increase in the provocative concentration of methacholine required to cause a 20% fall in $FEV_1$ ($PC_{20}$) after one puff of salbutamol. When selected by these criteria, a dose-response with increasing doses of bronchodilator on methacholine $PC_{20}$ can be easily demonstrated. This method has been employed to demonstrate bioequivalence of salbutamol pressurized metered-dose inhalers (MDIs)[7,10] and also dry powder inhalers.[11,12] In a crossover study design, approximately 51 subjects are required to demonstrate bioequivalence with a 90% CI of between 0.8 and 1.25.[7]

**Protection from exercise-induced bronchoconstriction** Exercise is a more physiological stimulus to induce bronchoconstriction than nonspecific bronchoprovocation agents such as methacholine. Therefore, demonstration of bioequivalence of bronchodilators in protecting against exercise-induced bronchoconstriction is an attractive study design which has not been investigated or extensively reported in the literature. Reasonable outcome measures to examine can be either the maximum per cent fall in $FEV_1$ after exercise, or the area under the per cent fall in $FEV_1$/time curve for 30 or 60 min after exercise. Since the former measurement is more reproducible,[13] it may have greater power in clinical trials than area under the curve (AUC) measurements.

Although exercise-induced bronchoconstriction can be attenuated by short-acting bronchodilators, no data has been published reporting the RP of two bronchodilators using this method. Two clinical trials in the levalbuterol development program compared levalbuterol (Xopenex® HFA MDI, 45 µg/actuation) to racemic albuterol (Proventil® HFA MDI, 90 µg/activation) using this protection from exercise-induced bronchoconstriction as the outcome variable of interest (data on file, Sepracor Inc.). In adults, where both aerosols were delivered through a spacer device, the RP of levalbuterol did not meet the above equivalence criteria, with the RP estimate and 90% CI being 0.49 (0.03, 2.84) for the primary pulmonary function outcome variable (percent decrease from visit post-dose/pre-challenge AUC $FEV_1$, and 0.68 (0.21, 1.80) for the key secondary outcome (maximum per cent decrease from visit post-dose/pre-challenge in $FEV_1$). Later in vitro laboratory characterization studies found that differential spacer retention of active drug accounted for this result (data on file, Sepracor Inc.) In children (aged 4–11 years), near optimal protection from a fall in $FEV_1$ was observed in both the levalbuterol HFA MDI and the Proventil HFA MDI groups after a single actuation of either drug. The absence of a dose-response relationship between the 1X, 2X, and 4X doses and the extent of bronchoprotection did not allow an RP analysis in

this trial, as this did not satisfy the analytic requirements for RP testing using slope estimates.[14] It should also be noted that these studies were not powered to make definitive conclusions between the treatment effects of levalbuterol vs. racemic albuterol.

In a cumulative dose design clinical trial in adults that also employed spacers, equivalent RP could also not be established within accepted CIs (RP = 0.69, 90% CI 0.33–1.38; data on file, Sepracor Inc.). In both this study and the study above comparing the brochoprotective effect of levalbuterol vs. racemic albuterol using exercise challenge in adults, the spacers were used incorrectly, therefore limiting the ability to interpret the results of these studies (data on file, Sepracor Inc.).

At least three challenges to the evaluation of bronchodilator bioequivalence are highlighted in these trials. First, an adequate sample size to evaluate bioequivalence is likely to depend upon the outcome selected and how reproducible the bronchodilator or bronchoprotection response is for that outcome. Second, the linear portion of the dose-response curve must be assessable and the range of dose evaluation must be able to access this range. If the lowest dose (e.g., a single actuation of an MDI) results in optimal bronchodilation or bronchoprotection for the bronchodilator agents being compared, a bioequivalence evaluation is not possible. Third, factors in the study design that could influence the delivery or inherent activity of the bronchodilator agents in the study can impact results. For example, in comparative bioequivalence trials that employ spacers, differences in spacer retention of drug due to charge, particle size distribution, particle geometry, or electrostatic properties of agents could influence drug delivery and bronchodilator performance. Relative bioequivalence for one outcome or experimental design does not, therefore, imply a similar result for a different outcome or set of experimental/study design circumstances.

Studies examining RP by protection against exercise-induced bronchoconstriction should utilize a crossover design to minimize between-person variablility when possible, as the dose-response relationship may have a shallow slope if the agents tested are effective even at low doses.

Other stimuli, such as mannitol or hyperosmolar saline, could also potentially be suitable for comparing the RP of bronchodilators.

**Cumulative vs. noncumulative design** Most published studies of bioequivalence of inhaled bronchodilators have employed a cumulative study design to construct dose-response curves of $FEV_1$.[6,8,15] There is some concern that the dose-response obtained by cumulative dosing of a bronchodilator may be different from that of a noncumulative dosing. A cumulative dosing may cause a steeper dose-response curve without a clear plateau response compared to the same dose delivered noncumulatively.[16] This may result in the failure to detect a difference in dose-potency between two bronchodilators even where a true difference exists. However, this was not true in the estimation of the RP of Proventil-HFA and Ventolin-CFC. The RP estimated from a cumulative bronchodilator study (RP 1.08; 90% CI 0.95–1.23) was comparable to the RP obtained by comparing the protective effect on methacholine-induced bronchoconstriction in a noncumulative study (RP 1.08; 90% CI 0.81–1.46).[7] The significance of this observation is that the model employed to estimate RP ought to produce a steep dose-response relationship for the outcome selected for comparison, whether it is cumulative or noncumulative design.

**Comparative assessments not included in bronchodilator bioequivalence testing** A comparison of RP by any of the methods described above inherently evaluates only the bronchodilator properties of the agents compared. These methods do not include comparisons of the tolerability or safety of compared agents, particularly with continued use over prolonged treatment periods, nor do they necessarily incorporate a comparison of the duration of action of the agents. Therefore, differences in the properties of the active drug substance or excipients in the tested formulations that impact important clinical outcomes may exist independent of the presence or absence of bronchodilation equivalence.

**Summary and conclusions** The RP of bronchodilators may be estimated by comparing either their bronchodilator or bronchoprotective properties. Bronchodilator studies are easier to perform and may better model the physiologic effect of many agents, including inhaled β-agonists. However, it may be difficult to demonstrate steep dose-response for these outcomes, except in cumulative study designs. Bioequivalence trials may be especially challenging when involving pressurized metered-dose inhalers, as a single actuation – the lowest feasible dose to include in the evaluation, may already produce bronchodilation that is at or near the plateau of the dose-response curve. Studies with shallow dose-response may require impractically large sample sizes to demonstrate bioequivalence. Although protection against exercise is a physiologically relevant outcome to demonstrate bioequivalence, demonstration of steep dose-response at clinically relevant doses may be problematic. Protection against bronchoconstriction induced by a direct inhaled stimulus like methacholine or histamine affords a reliable and practical method of comparing inhaled bronchodilators and estimating their RP. Inhalational bronchoprovocation testing allows for easier repeatability and quantitation of the stimulus necessary to produce a predetermined degree of bronchoconstriction, and the degree of protection afforded by the bronchoprotection agent. RP studies using adequate methodology are necessary

to compare long-acting bronchodilators and both short- and long-acting bronchodilators in patients who are also on inhaled corticosteroids.

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# Badanie równoważności klinicznej wziewnych leków rozszerzających oskrzela

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**STRESZCZENIE**

Nie istnieją wystandaryzowane metody wykazania biorównoważności *in vivo* wziewnych leków rozszerzających oskrzela. Najbardziej praktyczną metodą wykazania równoważności terapeutycznej *in vivo* jest ocena ich względnej mocy w badaniach skuteczności klinicznej, w których względną moc leków rozszerzających oskrzela można oszacować poprzez porównanie ich właściwości bronchodylatacyjnych lub zapobiegających skurczowi oskrzeli. Łatwiej jest przeprowadzić badania tych leków pod kątem działania rozszerzającego oskrzela; badania takie mogą lepiej odzwierciedlać efekt fizjologiczny wielu substancji, w tym β-agonistów wziewnych. Jednakże wykazanie wyraźnej zależności odpowiedzi od dawki w takich badaniach może być trudne (z wyjątkiem badań oceniających efekt kumulacyjny). Badania biorównoważności mogą stanowić szczególne wyzwanie w przypadku inhalatorów ciśnieniowych z dozownikiem, gdyż uwolnienie pojedynczej dawki – najmniejszej, której efekt da się ocenić – może wywołać rozszerzenie oskrzeli odpowiadające lub bliskie *plateau* krzywej dawka–efekt. Ocena zapobiegania skurczowi oskrzeli wywoływanemu przez bezpośredni bodziec wziewny, taki jak metacholina lub histamina, stanowi wiarygodną i praktyczną metodę porównania wziewnych leków rozszerzających oskrzela i oceny ich względnej mocy. Wziewne oskrzelowe próby prowokacyjne zapewniają większą powtarzalność i ocenę ilości bodźca koniecznej do wywołania skurczu oskrzeli o z góry określonym nasileniu oraz ocenę stopnia, w jakim dany lek zapobiega temu skurczowi. Badania względnej mocy leków przy wykorzystaniu odpowiedniej metodologii są konieczne do porównania długo i krótko działających leków rozszerzających oskrzela u chorych przyjmujących również kortykosteroidy wziewne.

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