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Title: Direct and indirect bronchoprovocation tests in dose-response studies of inhaled corticosteroids: Past, present and future directions

Short Running Title: Dose-response of inhaled corticosteroids

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Abstract:

Inhaled corticosteroids (ICS) are a mainstay of treatment in eosinophilic asthma. Many studies have explored the dose-response effect of different formulations of ICS through direct or indirect bronchoprovocation testing. Such studies are important for investigating efficacy and identifying the relative potency between formulations. However, lack of consistency in methods and designs has hindered the comparability of study findings. This review discusses current knowledge of the dose-response, or lack thereof, of different formulations of ICS through direct and indirect bronchoprovocation testing. The strengths and weaknesses of past studies inform recommendations for future methodological considerations in this field, such as utilizing a randomized double-blind crossover design, enrolling participants likely to respond to ICS therapy, and carefully selecting treatment durations and washout periods to assess incremental improvement in airway hyperresponsiveness while reducing the likelihood of a carryover effect.

Keywords: asthma; bronchoprovocation; dose-response; inhaled corticosteroid

Key Points for the Practicing Physician:

- Dose-response studies facilitate the determination of the relative potency of different inhaled corticosteroids
- Dose effects can be assessed through direct and/or indirect bronchoprovocation challenges in asthmatics with normal spirometry at rest
- Lack of standardization among dose-response studies has largely hampered efforts to accurately establish the relative potency of these drugs

- Standard methodological features (e.g. crossover design, more stringent inclusion criteria, longer treatment durations, washout periods between each dose change) can produce superior results that are more easily compared between studies

Introduction:

Inhaled corticosteroids (ICS) are instrumental for the treatment of asthma and are indicated for all levels of symptom severity. In 2019, the Global Initiative for Asthma (GINA) changed its recommendations for the treatment of mild eosinophilic asthma;¹ instead of short-acting beta agonist only, an ICS should be prescribed earlier for more effective symptom control.

Several ICS formulations are available, each being unique in many factors (e.g. receptor-binding properties, pharmacokinetics, systemic effects, deliver systems).² Dose-response (DR) studies assess safety and efficacy while facilitating comparative research on the relative potency of different drugs.³

While many studies have explored the dose-dependent effect of ICS on clinical outcomes, a lack of standardization in study design hampers efforts to better understand the ICS DR in asthma.⁴ For example, some studies used a dose-escalation design without washouts, which likely results in a duration effect rather than or in addition to a DR; a duration effect would result if a treatment at a stable dose produces increasing benefit with longer term use. Most dose-escalation studies of the ICS DR involved treatment with each dose for as little as 1-3 weeks, which may not have been sufficient time for any particular dose to reach the maximum effect; consecutive doses could then seem to produce a larger effect, which may be wholly in part due to a longer overall treatment duration allowing for the achievement of near or full maximum effect.

Many studies have focused on the impact of ICS on spirometry. The participant pool for DR studies typically consists of mild-to-moderate asthmatics with no/minimal baseline airflow obstruction; therefore, minimal changes in lung function may be small due to limited capacity for improvement. An arguably more clinically relevant endpoint would be the effect of ICS on direct and indirect bronchoprovocation.^{5,6} Such tests may be abnormal and responsive to treatment despite normal

spirometry; direct stimuli act on airway smooth muscle receptors to trigger bronchoconstriction while indirect stimuli act through intermediate pathways to stimulate inflammatory mediator release.⁷ Indirect challenges (e.g. allergen, adenosine monophosphate, exercise, mannitol) are more specific for eosinophilic airway inflammation and should thus better identify individuals who are likely to respond to anti-inflammatory treatment with ICS.⁷ However, such tests are typically dose-limited, which can pose a problem; if participants experience sufficient benefit from treatment, they may no longer achieve the target endpoint (e.g. 15% fall in FEV₁, forced expiratory volume in 1 second) within the dose limit of a given test (e.g. maximum 635mg cumulative dose of mannitol available). While direct challenges (e.g. methacholine) lack specificity for airway inflammation, these tests are not dose limited, they are easy to perform, and the results can be correlated with airway inflammation through additional tests such as of fractional exhaled nitric oxide (FeNO) or sputum eosinophil cell counts.

This review assesses research to date on the DR effect of different ICS using direct or indirect bronchoprovocation. Based on the strengths and shortcomings of past studies, standard methodological considerations are proposed so that future findings in this field may be easily compared and of superior quality in assessing the DR of available ICS.

Knowledge to date:

Relevant studies were identified through searches of PubMed and Cochrane Library using the keywords “inhaled corticosteroid”, “asthma”, “dose response”, and “bronchoprovocation”. The reference lists of relevant studies were then examined to identify other studies. To be included in this review, studies needed to explore a minimum of two doses of at least one ICS in asthmatics through direct and/or indirect bronchoprovocation testing.

The studies described were randomized and double-blind unless specified otherwise. All doses are reported as that delivered from the valve (i.e. from the inhaler mouthpiece to the mouth), not from the actuator (i.e. from the drug storage to the valve with device activation). For consistency, full daily doses are described. When possible, study results are reported as the dose-shift i.e. doubling

concentration/dose change in responsiveness to bronchoconstricting stimulus. For most direct and indirect challenges, the treatment effects are determined based on changes in the dose or concentration of stimulus needed to reach the target change in lung function; more specifically, these data are reported as the provocative dose (PD) or provocative concentration (PC) of a stimulus that causes a 15% (i.e. PD₁₅ or PC₁₅) or 20% (PD₂₀ or PC₂₀) fall in FEV₁.

Beclomethasone dipropionate (BDP) – Table 1

Direct bronchoprovocation

The only BDP study using histamine challenges was of single blind, crossover design and compared 500mcg/d and 1000mcg/d.⁸ Dose-shifts in histamine PC₂₀ from baseline were 0.6 and 1.7, respectively; only the 1000mcg/d dose-shift was statistically significant.

A dose-escalation study involving 500mcg/d followed by 1000mcg/d did not find a DR through methacholine challenge testing (MCT) despite a lack of washout between doses.⁹ Dose-shifts in methacholine (MCh) PD₂₀ were 1.3 and 1.4, respectively.

A parallel study used MCT to compare placebo with two nebulized BDP doses, 1600mcg/d and 3200mcg/d;¹⁰ estimated dose-shifts were 1.0 and 1.5, respectively. Only 3200mcg/d significantly increased MCh PC₂₀ from baseline, with no significant DR. Study power and washout from prescribed ICS use were not disclosed.

The DR of two formulations of BDP, Qvar® and Beclate®, was investigated using a crossover, dose-escalation design of 100mcg/d followed by 400mcg/d.¹¹ Both 400mcg/d treatments significantly shifted MCh PC₂₀ from pooled baseline data. Although there was no mention of a significant difference between doses, the dose shift results for 100 and 400mcg/d suggest a small DR with Beclate® (0.31 and 0.96, respectively) and no DR with Qvar® (0.56 and 0.69, respectively).

A non-randomized, non-blinded, dose-escalation study involved four consecutive treatments of placebo, 100mcg/d, 400mcg/d, and 800mcg/d.¹² All active treatments significantly increased MCh PC₂₀ without a significant difference between doses despite dose-shifts from placebo of 2.2, 4.0, and 4.6, respectively. The short treatments, dose-escalation design, and lack of washout suggest a duration effect with or without a DR.

An open-label dose-escalation study compared 200, 800, and 1600mcg/d.¹³ All doses significantly increased MCh PC₂₀ with dose-shifts of 1.1, 1.4, and 1.6, respectively. There was no DR.

Indirect bronchoprovocation

An abstract described a crossover study whereby dual allergen responders completed 1 baseline and 3 single-dose periods of 50mcg, 150mcg, or 450mcg immediately prior to allergen challenge.¹⁴ Increasing doses of BDP showed a trend towards greater inhibition of the late asthmatic response (LAR) but no significant DR on the early asthmatic response (EAR) or LAR. Conversely, a parallel, dose-escalation study with consecutive dosing intervals of 400, 800, and 1600mcg/d suggested a DR with respect to the EAR;¹⁵ a lack of disclosure of numerical data precluded further analysis of dose-dependent differences. Short washouts between challenges, lack of placebo control, and small sample sizes in both studies influence the strength of the findings.

A crossover study in children ages 6-15 investigated the BDP DR through exercise challenge.¹⁶ Participants received three treatments: placebo, 50mcg/d, and 100mcg/d. Both BDP doses significantly reduced the maximum % fall in FEV₁ after exercise (20.8% and 20.9% for 50mcg/d and 100mcg/d, respectively) versus placebo (27.9%) without a difference between doses.

Similar results were reported in asthmatic adults.¹³ This open-label, dose-escalation study involved three consecutive doses (200, 800, and 1600mcg/d). The number of positive exercise challenges post-treatment made analysis difficult; the limited data suggest no DR.

A parallel trial compared placebo, 50, 100, 200, and 500mcg/d through hypertonic saline challenges.¹⁷ Saline PD₁₅ results were 12.5mL, 11.0mL, 13.3mL, 8.4mL, and 13.2mL for placebo, 50, 100, 200, and 500mcg/d, respectively, showing no significant difference between doses. Loss of symptom control in 25 participants (6 from placebo, 5 from 50mcg/d, 8 from 100mcg/d, 4 from 200mcg/d, 2 from 1000mcg/d) resulted in a reduction in overall sample size.

Budesonide (BUD) – Table 2

Direct bronchoprovocation

A parallel study found that while 1600mcg/d and 3200mcg/d significantly increased histamine PD₂₀, there was no significant difference between doses.¹⁸ Dose-shifts were approximately 2.4 and 2.9, respectively. Washout from prescribed ICS was unclear.

Three parallel studies investigated the potential DR using 200 and 800mcg/d BUD. The first categorized participants into two groups, those with asthma symptoms for <12 months (“new asthma”) and those with asthma symptoms for >24 months (“established asthma”).¹⁹ Within each group, 40% were randomized to 200mcg/d, 40% to 800mcg/d, and 20% to placebo. Dose-shifts in histamine PD₂₀ for 200mcg/d and 800mcg/d, respectively, were 1.6 and 2.6 in the “new asthma” group and 1.0 and 2.6 in the “established asthma” group; only the “established asthma” group showed a significant DR. The second study involved treatment with 200 or 800mcg/d in recently diagnosed (<3 months) asthmatics.²⁰ Both doses significantly increased histamine PC₂₀ but no DR was observed; the maximum recorded dose-shifts were 1.0 with 200mcg/d and 1.5 with 800mcg/d. Results these two studies suggest a DR to ICS is less likely among newly-diagnosed asthmatics. The third study reported a significant DR through MCT with dose-shifts of 0.8 and 1.6 for 200 and 800mcg/d, respectively.²¹ Symptom duration was not disclosed.

One publication combined two studies identical in eligibility criteria and largely similar in methodology;²² the parallel study involved placebo, 100mcg/d, and 400mcg/d. The crossover study involved placebo and 1600mcg/d. There was no significant difference in MCh PC₂₀ with 100mcg/d or

400mcg/d compared to baseline with dose-shifts of 1.7 and 1.8, respectively; 1600mcg/d significantly increased MCh PC₂₀ by a dose-shift from baseline of 3.4.

An open-label, non-randomized, dose-escalation study also suggested that BUD exhibits a DR at higher doses, but a duration effect may have been observed.²³ Participants received sequential doses of 400mcg/d, 800mcg/d, and 1600mcg/d. MCh PD₂₀ did not differ between 400 and 800mcg/d, and both had significantly less effect than 1600mcg/d; dose-shifts were 1.4, 1.7, and 2.7, respectively.

Another dose-escalation study with 3 consecutive treatment periods with 800, 1600, and 3200mcg/d reported a linear DR relationship with MCh dose-shifts of 0.1, 0.4, and 0.9, respectively.²⁴

A crossover study comparing placebo, 200, 400, and 800mcg/d did not find a DR through MCT;²⁵ MCh dose-shifts from placebo were 0.8, 0.9, and 1.1, respectively.

Indirect bronchoprovocation

The previously described open-label, dose-escalation study²³ also investigated the BUD DR through adenosine monophosphate (AMP) challenge. While MCT results suggested a DR at higher doses, AMP showed a linear DR with dose-shifts of 2.5, 3.7, and 4.4 for 400, 800, and 1600mcg/d, respectively. A recent study also reported a significant DR when assessing several doses of three ICS through repeat AMP challenges;²⁶ the BUD dose-escalation arm of 5 consecutive, sequential periods (100, 400, 800, 1600, and 3200mcg/d) produced dose-shifts of 0.1, 0.7, 1.2, 1.8, and 2.5, respectively. These dose-shifts are significantly smaller than those noted above²³ despite this study's inclusion of a higher final dose, which may be due in part to differences in dose duration.

Dual allergen responders completed a crossover study involving 3 single-dose treatments: placebo, 200mcg, 800mcg.²⁷ Neither BUD dose significantly changed the EAR from placebo while both significantly attenuated the LAR. No difference was found between doses; the maximum % fall in FEV₁ during the LAR was 34.1% with placebo, 15.1% with 200mcg, and 11.2% with 800mcg. The short washout between allergen challenges may have produced a sequence effect.

A crossover trial of 4 treatment periods, placebo, 200, 400, and 800mcg/d, did not find a DR.²⁵ All doses significantly increased the allergen EAR PC₁₅ compared to placebo but dose-shifts of 1.3, 1.2, and 1.6, respectively, were not significantly different. A sequence effect trend was reported, which may have resulted from short washouts between challenges/treatments.

Two crossover dose-escalation studies found a DR when assessing BUD effectiveness through AMP challenges. One study entailed sequential doubling doses of 200, 400, and 800mcg/d.²⁸ There were 2 blocks using different devices, the Turbuhaler® and the Clickhaler®. A dose-dependent change in AMP PC₂₀ was found that formed a plateau at higher doses; 200mcg/d produced a less significant increase in AMP PC₂₀ compared to 400mcg/d and 800mcg/d, with no difference between the two higher doses. The only difference between devices was the shape of the DR curve; the Clickhaler® produced a mild linear trend (dose-shifts of 2.6, 3.2, and 3.5, respectively); the Turbuhaler® produced a plateau (dose-shifts of 2.7, 3.5, 3.4, respectively).

A single-blind, crossover, dose-escalation study included three blocks.²⁹ One block involved 1-week sequential dosing with 100, 400, and 1600mcg/d. Another block was identical except dosing was in 2-week intervals. The remaining block was 1600mcg/d for 2 weeks. The two dose-escalation blocks were statistically similar; both showed a significant dose-related increase in AMP PD₂₀ with dose-shifts of 1.5, 2.0, and 3.1, respectively, during the 1-week dosing regimen and 2.0, 2.7, and 4.0, respectively, during the 2-week dosing regimen. A duration effect is probable; the 1600mcg/d regimen produced dose-shifts of 2.1 and 3.7 at 1 and 2 weeks, respectively, which are both lower than the dose-escalation regimens.

Two exercise challenge studies in children produced different results; the first, a crossover study in children ages 6-15, featured 3 consecutive treatment periods with 100mcg/d, 200mcg/d, and 400mcg/d without washout.³⁰ The results showed a DR; the maximum % fall in FEV₁ from exercise was 55.4% at baseline, 25.7% with 100mcg/d, 20.1% with 200mcg/d, and 9.9% with 400mcg/d. The second study was of parallel design in children ages 7-16 and included four treatment groups:

placebo, 100mcg once daily, 200mcg once daily, and 100mcg twice daily.³¹ All three doses significantly reduced exercise-induced bronchoconstriction compared to placebo with no significant dose differences. The mean changes from baseline in maximum % fall in FEV₁ from exercise were 4.2%, 16.3%, 14.8%, and 12.8% with placebo, 100mcg once daily, 200mcg once daily, and 100mcg twice daily, respectively.

Ciclesonide (CIC) – Table 3

Direct bronchoprovocation

None identified.

Indirect bronchoprovocation

Three of five CIC studies did not report a DR. A crossover trial comparing placebo, 400mcg/d, and 1600mcg/d found dose-shifts in AMP PC₂₀ of approximately 2.8 for both doses, reflecting a flat DR curve.³² Similarly, a crossover study failed to show a DR when comparing placebo, 3 doses of CIC (400, 800, and 1600mcg/d), and 2 doses of another ICS.³³ AMP dose-shifts from placebo were 2.2, 2.3, and 2.8 for 400, 800, and 1600mcg/d CIC, respectively. A third crossover study compared placebo, 50mg, and 100mcg CIC through allergen challenges;³⁴ although only the 100mcg dose significantly reduced the maximum % fall in FEV₁ during the EAR compared to placebo, both doses significantly reduced the maximum % fall in FEV₁ during the LAR and there were no significant differences between doses for either outcome. The maximum % falls in FEV₁ were 30.4%, 28.2%, and 23.6% during the EAR and 24.0%, 13.3%, and 10.7% during the LAR for placebo, 50mcg, and 100mcg, respectively.

A parallel study including placebo, 100mcg/d, 400mcg/d, and 1600mcg/d had a crossover component whereby participants completed one arm at a randomized dose and one placebo arm.³⁵ Repeat AMP challenges showed a significant dose-dependent increase in AMP PC₂₀; while the lowest dose did not statistically increase AMP PC₂₀ versus placebo, the two higher doses did. Dose-shifts from placebo were 1.6, 2.0, and 3.4, respectively.

In a parallel study, participants ages 12-30 were randomized to complete a crossover arm of 50mcg/d and 200mcg/d, or a crossover arm of 100mcg/d and 400mcg/d.³⁶ All doses significantly decreased the maximum % fall in FEV₁ from exercise compared to baseline: 21.8% with 50mcg/d, 19.1% with 100mcg/d, 18.2% with 200mcg/d, and 13.4% with 400mcg/d. Results following doses of 50mcg/d and 400mcg/d differed significantly while all other dose differences were insignificant.

Fluticasone propionate (FP) – Table 4

Direct bronchoprovocation

Atopic asthmatics with baseline sputum eosinophilia >2.5% completed a single-cohort, single-blind, dose-escalation study comparing 50, 100, 200, and 400mcg/d.³⁷ Only the two highest doses significantly changed MCh PC₂₀ from baseline. Dose-shifts were 0.2, 0.3, 0.8, and 1.3, respectively, which suggests a small dose and/or duration effect.

A parallel trial of children ages 6-14 that included placebo, 200mcg/d, and 500mcg/d also found a DR.³⁸ After 6 weeks, the placebo group was re-distributed to the two active treatment groups for another 18 weeks. MCh PD₂₀ dose-shifts from baseline in were 1.1 and 2.1 at 6 and 24 weeks, respectively, for the 200mcg/d group and were 1.6 and 3.3 at 6 and 24 weeks, respectively, for the 500mcg/d group.

In atopic asthmatics, a parallel study including 100, 500, and 2000mcg/d FP found no significant DR.³⁹ Although only the 500mcg/d dose was reported to significantly change the mean histamine PC₂₀, graphed data do not suggest a significant change with any dose. The lack of reported numerical data precluded further analysis of the dose-shifts. In comparison, a dose-escalation study with similar doses found a small DR in a parallel study comparing BUD and FP.²⁴ The FP group received sequential doses of 500, 1000, and 2000mcg/d; dose-shifts in MCh PD₂₀ were 0.1, 0.4, and 0.9, respectively. In smaller doses, an open-label dose-escalation trial of 100, 400, and 800mcg/d FP produced a flat DR curve through MCT;¹³ dose-shifts were 1.3, 1.6, and 1.5, respectively.

A parallel study found no DR when comparing 100 and 1000mcg/d.⁴⁰ Both doses significantly increased MCh PC₂₀ by dose-shifts of 1.3 and 1.5, respectively. The only difference between doses was the longer sustained effect after withdrawal from the higher dose during the placebo run-out.

A parallel study of individuals ages ≥ 12 included treatment with placebo (1% lecithin), 100mcg/d FP (1% lecithin), 200mcg/d FP (1% lecithin), and 200mcg/d (10% lecithin).⁴¹ Neither 1% lecithin dose significantly increased the MCh PC₂₀ compared to placebo; the dose-shifts from placebo were 0.5 and 0.4 for 100mcg/d and 200mcg/d, respectively. Again, no DR was found.

A flat DR curve was reported based on airway hyperresponsiveness (AHR) to MCh in a parallel study that grouped participants into daily doses of 500mcg and 2000mcg.⁴² Dose-shifts were 1.7 and 2.0 for the low and high doses, respectively.

A lack of DR was also reported in a crossover, single-blind, dose-escalation study.⁹ Two treatment periods, one with FP and one with BDP, involved consecutive dosing with 500mcg/d followed by 1000mcg/d. Both doses of FP significantly increased MCh PD₂₀ without a significant difference between doses (dose-shifts of 1.3 and 1.4 for 500 and 1000mcg/d, respectively). A significant sequence effect was noted.

Indirect bronchoprovocation

Similar to the MCT findings noted above,⁴² a flat DR curve was found from 500mcg/d and 200mcg/d with dose-shifts of 3.5 and 4.0, respectively. Likewise, there was a lack of DR in both MCT and exercise challenge results;¹³ sequential dosing with 100, 400, and 800mcg/d resulted in dose-shifts outside the range of a positive challenge, reducing the sample size below 15.

While there was a small but significant DR with MCT in children (see above), the same was not true with exercise challenges.³⁸ The maximum % fall in FEV₁ from exercise was 10.3% at 6 weeks and

9.7% at 24 weeks with 200mcg/d, and 11.4% at 6 weeks and 12.3% at 24 weeks with 500mcg/d; both doses significantly reduced exercise-induced bronchoconstriction by similar magnitudes.

A crossover trial found a trend toward a DR.⁴³ Participants self-administered a total of 3 inhalations, each 12 hours apart, followed by AMP challenge for each of 4 treatments: placebo, 100mcg/inhalation, 250mcg/inhalation, and 1000mcg/inhalation. The dose-shifts were 2.4, 2.2, and 3.2 for 100, 250, and 1000mcg/inhalation, respectively. Another crossover study compared placebo, 3 doses of CIC, and 2 doses of FP (1000 and 2000mcg/d).³³ The dose-shifts in AMP PC₂₀ for the FP treatment periods were 2.8 and 2.9 for 1000 and 2000mcg/d, respectively. Neither ICS produced a significant DR.

Only one AMP challenge study reported a significant DR with FP in a recent incomplete crossover, 2-period, dose-escalation trial.²⁶ The FP dose-escalation arm involved sequential doses: 50, 200, 500, 1000, and 2000mcg/d. The dose-shifts were 0, 0.4, 1.1, 1.7, and 2.3, respectively.

A crossover study comparing 100 and 500mcg/d required participants to have an elevated FeNO (>30ppb) at screening.⁴⁴ Both doses resulted in a significant increase in mannitol PD₁₅ but no significant difference between doses with dose-shifts of 1.6 and 2.0, respectively.

Another mannitol challenge study also found no significant DR.⁴⁵ This parallel study included 200 and 1000mcg/d. Both doses significantly increased mannitol PD₁₅ but, like the prior study,⁴⁴ the doses did not differ significantly with dose-shifts of 3.2 for 200mcg/d and 2.5 for 1000mcg/d.

Mometasone furoate (MF) – Table 5

Direct bronchoprovocation

None identified.

Indirect bronchoprovocation

A crossover study compared placebo, 100mcg/d, 200mcg/d, and 800mcg/d MF through allergen challenges in dual allergen responders.⁴⁶ Although both the EAR and LAR were significantly decreased following all doses of MF, only the LAR showed a DR; the maximal % fall in FEV₁ from allergen was 23.5% with placebo, 12.3% with 100mcg/d, 11.0% with 200mcg/d, and 5.9% with 800mcg/d.

Two other studies, one with AMP and one with mannitol, did not find a significant DR. The former, a crossover trial with placebo, 100mcg/d, and 200mcg/d, reported AMP dose-shifts from placebo of 2.8 and 3.1, respectively.⁴⁷ The latter, a parallel study of participants ages ≥ 12 , involved placebo and five active treatments: two MF-only treatments of 400mcg/d (metered dose inhaler) and 400mcg/d (dry powder inhaler), and three MF/formoterol combinations of 200/20, 400/20, and 800/20mcg/d.⁴⁸ This is the only dose-ranging study identified that used a combination treatment with different ICS doses and consistent doses of long-acting beta agonist (LABA). An elevated FeNO (>30 ppb) and sputum eosinophil counts $>3\%$ were required at screening. Several participants had negative mannitol challenges post-treatment, which influenced the feasibility of assessing for a DR. Only 200/20mcg/d MF/formoterol produced a significant improvement in mannitol PD₁₅ from placebo.

Other ICS – Table 6

Direct bronchoprovocation

None identified.

Indirect bronchoprovocation

Three ICS were studied in a parallel, dose-escalation study. In one study, participants received one of the following dose-escalation treatments with allergen challenge before each dose step-up: triamcinolone acetonide 800-1600-3200mcg/d, flunisolide 1000-2000-4000mcg/d, or BDP at three different doses (BDP previously discussed).¹⁵ The data, reported in graph form only, showed a DR trend with all three ICS; lack of numerical data precluded further analysis of the attenuation of the EAR.

One AMP challenge study included a fluticasone furoate (FF) dose-escalation arm at sequential doses of 25, 100, 200, 400, and 800mcg/d.²⁶ Dose-shifts in AMP PC₂₀ from baseline were 1.1, 2.4, 2.9, 3.3, and 3.5, respectively, which suggests that the DR develops a plateau. The other two ICS from this study, BUD and FP, both showed significant DRs without the development of a plateau (discussed above).

Discussion:

Methodological Considerations:

An ideal DR study would feature the following: a randomized double-blind placebo-controlled crossover design, participant selection based on significant airway eosinophilia at baseline, repeat measures of airway inflammation and AHR throughout treatments, ≥ 2 doses within a clinically-relevant range both delivered using the same type of device with the same dose unit, treatment and washout periods of ≥ 4 weeks, safety measures as a secondary endpoint, and the presentation of data in the form of dose-shifts (or maximum % fall in FEV₁ post-exercise or post-allergen) from baseline or placebo. The following sections discuss these features to justify their inclusion in a well-designed DR study.

Study Design

The fundamental dose-ranging study designs are parallel, crossover, and dose-escalation, each with strengths and weaknesses (Table 7). Many studies have utilized a parallel design without enrolling the larger sample needed compared to a crossover design to provide adequate power. Responsiveness to ICS is highly variable and so a consistent participant pool for all treatment arms is ideal. If using a parallel design, a placebo control arm is especially important and randomization into study groups should be stratified to ensure equivalent representation between groups with respect to duration and severity of asthma, degree of airway eosinophilia, and past/present ICS use. One main disadvantage of a crossover design is the longer study duration due to combined treatment lengths and washouts; nonetheless, this design provides strong study power with smaller samples, studies can still be

designed to avoid participants' allergy seasons, and confounding factors among participants are minimized. The chief concern with dose-escalation studies is the question of the extent to which a duration effect, rather than a DR, influences results. DR studies in general should be double-blind and placebo-controlled unless safety among severe asthmatic participants is a concern.

Participant Selection

It is important to enrol individuals likely to benefit from ICS treatment i.e. asthmatics with significant resting airway eosinophilia. Specific criteria to address this include screening for sputum eosinophils >3% and/or elevated FeNO (>25ppb at minimum, >50ppb to increase the likelihood of eosinophilic inflammation and response to ICS).⁴⁹ These are also valuable study endpoints to assess dose differences in reducing airway inflammation, which can then be compared with changes in AHR to direct/indirect stimuli. Although almost half of the studies reviewed included one of these proposed endpoints, few had specific inclusion criteria regarding airway inflammation, meaning that individuals with limited room for improvement with respect to airway inflammation may have been included.

Past inclusion criteria regarding ICS use have ranged from requiring full steroid naïveté to long washouts of ≥ 4 weeks or zero washout. ICS treatment takes time to reach full effect and to wear off when discontinued. To isolate findings strictly to the test ICS doses, any previous ICS would need to be discontinued for several weeks. A standard washout/run-in period from prescribed ICS prior to study enrollment would help to ensure that participants are more comparable at baseline. However, there are important ethical concerns to consider, particularly in more severe asthmatics who rely on regular ICS therapy for symptom control. Regardless of ICS use criteria, studies including participants of different ICS use histories should stratify randomization to equally divide participants between different randomization schemes.

Direct or Indirect Bronchoprovocation

Since the primary mechanism of action of ICS is to reduce airway inflammation, indirect bronchoprovocation may be more clinically relevant due to the induction of inflammatory mediator

release; direct bronchoprovocation (e.g. MCT) is only weakly correlated with eosinophilic airway inflammation.⁷ Regardless, significant DR relationships have been reported using both bronchoprovocation methods. Perhaps more importantly, ICS dose-ranging studies should consider airway inflammation in some form, be it through repeated sputum eosinophil cell counts or FeNO measures and/or through indirect bronchoprovocation. Furthermore, the testing method should be chosen carefully by considering potential shifts in AHR outside the range of a positive test; this has posed a problem in the past such as when a study treatment shifted many participants' response outside the range of a positive exercise challenge,¹³ which precluded statistical analysis of the possible DR.

Dosing

A starting point for determining the dose levels to include in a DR study is to consider doses currently routinely prescribed in clinical practice. Alternatively, the GINA outlines proposed low-medium-high doses for the ICS formulations currently available.⁵⁰ Past DR and safety studies could also be helpful.

There are efficacy and adherence considerations when choosing dosing frequency. Several studies have shown equivalent efficacy between once- and twice-daily dosing with various ICS.^{51–54} Patients have also been reported to exhibit better treatment adherence with a lower dosing frequency.^{55–57} It is recommended that dosing frequency be once-daily at the same time of day.

It could be worthwhile, albeit challenging, to complete DR studies using combination ICS/LABA inhalers. Both ICS monotherapy or low-dose BUD/formoterol combination treatment are encouraged even among mild asthmatics for improving symptom control and decreasing exacerbation risk. However, additional considerations would be necessary. For example, like previously done,⁴⁸ such studies would need to keep the LABA dose consistent. Moreover, a more complex methodology would be required to differentiate the effect of the combination versus each individual treatment component; repeat bronchoprovocation testing during the treatment period would reflect the effects of the combined ICS/LABA; repeat bronchoprovocation tests at different times after discontinuation may help to distinguish ICS-induced and LABA-induced effects. The LABA should theoretically be

completely metabolized within a matter of 3-4 days while the ICS should have lost effect within ≥ 2 weeks.

Device

Devices should be identical when comparing multiple doses of a particular ICS to maintain dose delivery specifications, which could otherwise bias the magnitude of clinical effect recorded. To best minimize potential discrepancies in the delivered dose between devices of the same type, devices with the same unit dose should be used for all treatments.² Devices that provide an objective dose counter are helpful for easily monitoring treatment adherence.

Treatment Length

Various treatment lengths have been used in ICS dose-ranging studies from a single dose to ≥ 12 weeks. Most reviewed studies only measured changes in AHR at the beginning and end of a treatment period and did not explore changes over the course of the treatment; two studies that performed repeat direct/indirect challenges during the treatment period found either that what appeared to be a dose difference initially ultimately became insignificant over a few weeks or that doses that had provided equivalent effects initially began to show a DR after 2+ weeks.^{36,40} It is thus advisable to study different doses over several weeks to avoid missing a DR or to incorrectly report a DR that would likely disappear with longer term use. Treatment durations can significantly impact the feasibility of crossover studies and so there must be a balance between the number of treatment periods, the required washout between each period, and the availability of participants outside their allergy seasons.

Washouts

Dose-ranging studies should employ a washout between all treatments and dose changes to avoid attributing differences to a DR effect when more likely a duration effect. The results from dose-escalation studies are most likely to be biased by a carryover effect. Baseline measures before each treatment period must consider airway inflammation and not only lung function, as it is the former that is likely to still be influenced by past ICS treatment for several weeks after treatment cessation.

Secondary Endpoints

For the sake of completeness in assessing the clinical and laboratory effects of different ICS doses, studies should be carefully designed to include several secondary endpoints. Past ICS DR studies greatly varied in terms of the secondary endpoints included, which influenced the correlations that could be made between changes in AHR and clinical outcomes such as changes in symptom frequency and severity. Airway inflammation can be followed through repeat FeNO measures and/or sputum and peripheral blood eosinophilia. Patient safety, which guides treatment dosing decisions, can be explored by reporting adverse effects and/or 24-hour urine cortisol levels. Although daily records of AM/PM symptoms and SABA use add to the work for participants, this information can provide clinically relevant information and may also help to explain discrepancies in data, for example, by facilitating recollection of potential allergen exposures and/or other events that may have influenced AHR.

Presentation of Data

The statistical significance from the treatments compared to baseline or placebo is relevant but it is also helpful to report numerical data in the form of dose-shifts (or maximum % fall in FEV₁ post-exercise or post-allergen) from baseline or placebo. By standardizing the manner in which dose-ranging study findings are reported, the results can be more easily compared to better understand the DR of various ICS. Several reviewed studies only reported p-values and raw data, which alone do not illustrate the clinical benefit or lack thereof from treatment with different doses. Power calculations should be provided to validate the strength of the results and were markedly absent in some studies.

Concluding Remarks:

Although many studies have explored the DR of currently available ICS through direct and indirect bronchoprovocation testing, the heterogeneity of methodologies impedes the accurate comparison of findings and the establishment of relative potencies between ICS. Dose-ranging studies are inherently difficult to design and complete given the number of variables and the duration of treatment involved. Nonetheless, this topic warrants further investigation through high quality research featuring elements

outlined in this review such as a crossover design, participant selection based on airway eosinophilia, and adequate washout periods between treatment arms rather than a dose escalation design.

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Tables:

Table 1. Beclomethasone dipropionate bronchoprovocation dose-response studies

Reference	Sample Size	Study Design	Doses (mcg)	Treatment Length per Dose (Washout) [†]	Drug Delivery Device	Placebo Control? (Y/N)	DR? (Y/N)	Washout from prior ICS use
Carpentiere et al ⁸	10	Crossover HC	500, 1000	3wks (3wks)	MDI	N	Y	None
Currie et al ⁹	20	Dose-escalation MCT	500, 1000	3wks	CFC-free MDI	N	N	7-14d
Bousquet et al ¹⁰	40	Parallel MCT	1600, 3200	3wks	Nebulizer or MDI+ spacer	Y	N	Unknown
Fardon et al ¹¹	15	Crossover Dose-escalation MCT	100, 400	2wks (1wk)	MDI+ spacer	N	N	Dose-tapering only
Silkoff et al ¹²	15	Dose-escalation MCT	100, 400, 800	1wk	MDI	Y	N	4wks
Szeffler et al ¹³	15	Dose-escalation MCT, EC	200, 800, 1600	6wks	MDI + spacer	N	N	6mo
Marshik et al ¹⁴	6	Crossover AC	50, 150, 450	Single dose (1wk)	MDI	N	N	Unknown
McCubbin et al ¹⁵	9	Dose-	400,	1wk	MDI	N	Y	6mo

		escalation	800,					
		AC	1600					
Petersen et al ¹⁶	25	Crossover	50,	4wks	Autohaler™	Y	N	2wk
		EC	100	(1wk)				
Jones et al ¹⁷	65	Parallel	50,	8wks	Autohaler™	Y	N	≤6wks
		HSC	100,					
			200,					
			500					

Abbreviations: AC, allergen challenge; DR, dose-response; EC, exercise challenge; HC, histamine challenge; HSC, hypertonic saline challenge; ICS, inhaled corticosteroid; MCT, methacholine challenge testing; MDI, metered dose inhaler;

† Washout durations were only featured in studies with a crossover component

Table 2. Budesonide bronchoprovocation dose-response studies

Reference	Sample Size	Study Design	Doses (mcg)	Treatment Length per Dose (Washout)†	Drug Delivery Device	Placebo Control? (Y/N)	DR? (Y/N)	Washout from prior ICS use
Reddel et al ¹⁸	61	Parallel HC	1600, 3200	8wks	Turbuhaler®	N	N	Unknown
Selroos et al ¹⁹	81	Parallel HC	200, 800	12wks	Turbuhaler®	Y	Y	ICS-naïve
Tukiainen et al ²⁰	97	Parallel HC	200, 800	12wks	Turbuhaler®	N	N	ICS-naïve
Kraan et al ²¹	30	Parallel MCT	200, 800	8wks	Not specified	N	Y	5wks
Jatakanon et al ²²	21 parallel 10 crossover	Parallel or crossover (2 studies) MCT	100, 400 (parallel) 1600 (crossover)	4wks (4wks)	Turbuhaler®	Y	Y	ICS-naïve
Wilson & Lipworth ²³	26	Dose-escalation MCT, AMP	400, 800, 1600	3wks	Turbuhaler®	N	Y	10d

Nielsen & Dahl ²⁴	33	Dose-escalation MCT	800, 1600, 3200	2wks	Turbuhaler®	N	Y	None
Swystun et al ²⁵	12	Crossover MCT, AC	200, 400, 800	1wk (6d)	Turbuhaler®	Y	N	4wks
Daley-Yates et al ²⁶	18	Dose-escalation AMP	100, 400, 800, 1600, 3200	1wk	Turbuhaler®	Y	Y	4wks
Kidney et al ²⁷	10	Crossover AC	200, 800	Single dose (1wk)	Turbuhaler®	Y	N	4wks
Lipworth et al ²⁸	27	Crossover Dose-escalation AMP	200, 400, 800	2wks (1-3wks)	Clickhaler® or Turbuhaler®	N	Y	None
Phillips et al ²⁹	21	Crossover Dose-escalation AMP	100, 400, 1600	1wk/dose or 2wk/dose or 1600mcg for 2wks (3wks)	Turbuhaler®	N	Y	4wks
Pedersen & Hansen ³⁰	19	Crossover EC	100, 200, 400	4wks (none)	Nebuhaler	N	Y	ICS-naïve
Jónasson et al ³¹	57	Parallel EC	100, 200 (100 BID), 200 (OD)	12wks	Turbuhaler®	Y	N	2mo

Abbreviations: AC, allergen challenge; AMP, adenosine monophosphate challenge; BID, twice day; DR, dose-response; EC, exercise challenge; HC, histamine challenge; ICS, inhaled corticosteroid; MCT, methacholine challenge testing; OD, once daily;

† Washout durations were only featured in studies with a crossover component

Table 3. Ciclesonide bronchoprovocation dose-response studies

Reference	Sample Size	Study Design	Doses (mcg)	Treatment Length per Dose	Drug Delivery Device	Placebo Control? (Y/N)	DR? (Y/N)	Washout from prior ICS use
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(Washout) [†]								
Erin et al ³²	21	Crossover AMP	400, 1600	1wk (4-6wks)	MDI	Y	N	4wks
Derom et al ³³	26	Crossover AMP	400, 800, 1600	9d (3-12wks)	MDI	Y	N	4wks
Gauvreau et al ³⁴	21	Crossover AC	50, 100	1wk (3wks)	MDI	Y	Y	4wks
Taylor et al ³⁵	29	Parallel AMP	100, 400, 1600	2wks	Cyclohaler TM	Y	Y	ICS-naïve
Subbarao et al ³⁶	24	Parallel & Crossover EC	50 & 200 or 100 & 400	3wks (3wks)	MDI	N	Y	ICS-naïve

Abbreviations: AMP, adenosine monophosphate challenge; DR, dose-response; EC, exercise challenge; ICS, inhaled corticosteroid; MDI, metered dose inhaler;

[†] Washout durations were only featured in studies with a crossover component

Table 4. Fluticasone propionate bronchoprovocation dose-response studies

Reference	Sample Size	Study Design	Doses (mcg)	Treatment Length per Dose (Washout) [†]	Drug Delivery Device	Placebo Control? (Y/N)	DR? (Y/N)	Washout from prior ICS use
Kelly et al ³⁷	14	Dose-escalation MCT	50, 100, 200, 400	1wk	MDI+ Aerochamber	Y	Y	4wks
Hofstra et al ³⁸	37	Parallel MCT, EC	200, 500	18wks	Volumatic TM	Y	Y(MCT) N(EC)	4mo
O'Sullivan et al ³⁹	36	Parallel HC	100, 500, 2000	2wks	Volumatic TM	N	N	ICS-naïve
Nielsen & Dahl ²⁴	33	Dose-escalation MCT	500, 1000, 2000	2wks	Diskhaler	N	Y	None
Szeffler et al ¹³	15	Dose-escalation	100, 400, 800	6wks	MDI+ spacer	N	N	6mo

MCT, EC								
Gershman et al ⁴⁰	24	Parallel MCT	100, 1000	6wks	Ellipse™	N	N	6wks
Noonan et al ⁴¹	129	Parallel MCT	100, 200	8wks	MDI	Y	N	ICS-naïve
Meijer et al ⁴²	120	Parallel MCT, AMP	500, 2000	2wks	Diskhaler	N	N	Dose-tapering only
Currie et al ⁹	20	Dose-escalation MCT	500, 1000	3wks	Evohaler	N	N	7-14d
Ketchell et al ⁴³	12	Crossover AMP	100, 250, 1000	3x each dose (1wk)	Accuhaler	Y	N	4wks
Derom et al ³³	26	Crossover AMP	1000, 2000	9d (3-12wks)	MDI	Y	N	4wks
Daley-Yates et al ²⁶	20	Dose-escalation AMP	50, 200, 500, 1000, 2000	1wk	Diskus®	Y	Y	4wks
Anderson et al ⁴⁴	21	Crossover MAN	100, 500	2wks (2wks)	Evohaler	N	N	2wks
Baraket et al ⁴⁵	22	Parallel MAN	200, 1000	7wks	MDI+ spacer	N	N	2mo

Abbreviations: AMP, adenosine monophosphate challenge; DR, dose-response; EC, exercise challenge; HC, histamine challenge; ICS, inhaled corticosteroid; MAN, mannitol challenge; MCT, methacholine challenge testing; MDI, metered dose inhaler;

† Washout durations were only featured in studies with a crossover component

Table 5. Mometasone furoate bronchoprovocation dose-response studies

Reference	Sample Size	Study Design	Doses (mcg)	Treatment Length per Dose (Washout)†	Drug Delivery Device	Placebo Control? (Y/N)	DR? (Y/N)	Washout from prior ICS use
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Inman et al ⁴⁶	11	Crossover AC	100, 200, 800	6d (3wks)	DPI	Y	Y	ICS-naïve
Holgate et al ⁴⁷	13	Crossover AMP	100, 200	2wks (4wks)	DPI	Y	N	1mo
Nolte et al ⁴⁸	92	Parallel MAN	200, 400, 800	2wks	MDI	Y	N	2wks

Abbreviations: AC, allergen challenge; AMP, adenosine monophosphate challenge; DPI, dry powder inhaler; DR, dose-response; ICS, inhaled corticosteroid; MAN, mannitol challenge; MDI, metered dose inhaler;

† Washout durations were only featured in studies with a crossover component

Table 6. Other ICS bronchoprovocation dose-response studies

Reference	ICS	Sample Size	Study Design	Doses (mcg)	Treatment Length per Dose	Drug Delivery Device	Placebo Control? (Y/N)	DR? (Y/N)	Washout from prior ICS use
McCubbin et al ¹⁵	Triamcinolone acetonide	8	Dose-escalation AC	800, 1600, 3200	1wk	MDI	N	Y	6mo
McCubbin et al ¹⁵	Flunisolide	9	Dose-escalation AC	1000, 2000, 4000	1wk	MDI	N	Y	6mo
Daley-Yates et al ²⁶	Fluticasone furoate	19	Dose-escalation AMP	25, 100, 200, 400, 800	1wk	Ellipta®	Y	Y	4wks

Abbreviations: AC, allergen challenge; AMP, adenosine monophosphate challenge; DR, dose-response; ICS, inhaled corticosteroid; MDI, metered dose inhaler;

† Washout durations were only featured in studies with a crossover component

Table 7. Comparison of fundamental methodologies for dose-response studies

	Advantages	Disadvantages
Crossover	Fewer confounders (consistent participant pool)	Longer study duration Adequate washouts required

	Each participant receives all doses Smaller sample size required Allow within-subject comparisons	Possible carryover effect
Parallel	Shorter study duration No carryover effect	Higher sample size required Confounding factors among participants No within-subject comparisons
Sequential Dose-Escalation	No washouts i.e. faster Each participant receives all doses	Duration effect Carryover effect Single cohort studies not double blind