Beclomethasone Has LesserSuppressive Effects onInflammation and AntibacterialImmunity Than Fluticasone orBudesonide in ExperimentalInfection Models

To the Editor:
Inhaled corticosteroids (ICS) are mainstay therapies inCOPD but are consistently linked with increasedpneumonia susceptibility. There is speculationregarding possible intraclass differences in pneumoniarisk between ICS agents, with some studies suggestingthat budesonide and beclomethasone dipropionate(BDP) confer lower pneumonia risk thanfluticasone propionate (FP).1-3 This has not been consistentlyshown4,5 and remains controversial. In the absence ofhead-to-head comparator trials, it is impossible toconclusively ascertain intraclass differences inpneumonia propensity. No previous studies havecompared the relative potential of these three ICSagents to impair host defense in experimentalinfection models, and mechanisms underlying anypotential differential effects on pneumoniasusceptibility are unknown. The agents differ in termsof glucocorticoid receptor affinity, solubility, andantiinflammatory potency,6 and thus they may have differing abilities to impair critical components ofantimicrobial host defense. We have recently reportedthat FP can impair epithelial control of thepneumonia-causing pathogen Streptococcus pneumoniae,mechanistically through inhibition of theantimicrobial peptide (AMP) cathelicidin.7 Usingexperiments in human cells and mouse infectionmodels, we performed a head-to-head comparison ofthe effects of the major ICS agents used in COPD oninnate immunity.

Methods
BEAS2B airway epithelial cells (AECs) were treated with FP,budesonide, and BDP (Sigma-Aldrich) at 0.1- to 1,000-nMconcentrations or vehicle dimethyl sulfoxide in 10% RPMImedium at 37°C for 1 hour before S pneumoniae D39 infection(1 × 10⁶ CFU/mL). In separate studies, 6-week-old female wild-type C57BL/6 mice were treated intranasally under isofluraneanesthesia with 20 μg FP, budesonide, BDP, or vehicle andinfected with 5 × 10⁷ CFU S pneumoniae D39 or PBS control.7Pneumococcal loads were quantified in homogenized lung tissueor cell supernatants and immune or inflammatory mediatorsmeasured in BAL or cell supernatants by enzyme-linkedimmunosorbent assay (ELISA). For in vitro experiments,conditions were run in triplicate wells and experiments repeatedthree times independently, with mean data from combinedexperiments analyzed by one-way analysis of variance withBonferroni’s multiple comparison test with use of the BrownForsythe test to confirm equality of group variances. Experimentsin mice involved five animals/group, and data shown arerepresentative of two independent experiments, analyzed usingKruskal-Wallis test with Dunn’s multiple comparison test.

Results
We initially evaluated antiinflammatory effects ofequimolar concentrations (0.1-1,000 nM) of FP,budesonide, and BDP in AECs infected with S pneumoniae. Previous studies of ICS administration inhumans show that a single inhaled dose of FP results inapproximately 10 nM lung tissue concentration.9 Because FP is reported to be approximately twofoldmore potent than budesonide or BDP,6 1- and 10-nM doses capture the full range of clinically relevant tissueconcentrations of all three agents encountered in vivo.FP and budesonide (at concentrations of 1 nM andhigher) suppressed induction of the pro-inflammatorycytokines IL-6 and chemokine (C-X-C motif) ligand 8/IL-8, with BDP only having significant effects at 10 nMor higher (Fig 1A). To confirm these effects in vivo, 20 μg of each ICS was administered in S pneumoniaeinfected mice. There was no difference in lungglucocorticoid receptor activation between the threeagents at this dose (Fig 1B). Significant suppression ofIL-6, tumor necrosis factor, and IL-1β and theneutrophil chemokine CXCL2/MIP-2 was only observedfor FP and budesonide (Fig 1B). All three ICSsignificantly reduced airway neutrophil recruitment (Fig1B). Combined, these data indicate that FP andbudesonide and, to a lesser extent, BDP can suppressinflammation during bacterial infection.

We next evaluated effects of ICS on bacterial burden inAECs. FP and budesonide, at concentrations of 1 nM or
higher, increased bacterial loads at 24 hours, with significant effects only observed for BDP at the highest concentration (1,000 nM) (Fig 2A). Previously, we reported that FP increases bacterial loads via suppression of the antimicrobial peptide (AMP) cathelicidin, an effect that occurs independently of other immune suppression and likely mediates ICS-related pneumonia risk in COPD.7 Accordingly, FP and
Budesonide suppressed epithelial human cathelicidin antimicrobial protein-18/LL-37 (hCAP-18/LL-37) induction by *S pneumoniae* at concentrations of 1 nM or higher, whereas BDP again only had effects at the maximal 1,000-nM concentration (Fig 2A). Similar effects were observed in vivo, with increased lung bacterial loads and reduced induction of the cathelicidin-related AMP (mouse ortholog) observed...
when FP or budesonide but not BDP was administered in S pneumoniae-infected mice (Fig 2B).

Discussion

Our experimental studies indicate an intra-class differential effect of ICS agents on antibacterial immunity. All three ICS agents could suppress inflammation during bacterial infection; however, at clinically relevant concentrations of 1 and 10 nM, only FP and budesonide inhibited cathelicidin (an AMP implicated in ICS-related pneumonia) and increased bacterial loads. Because BDP also exhibited lesser antiinflammatory effects, this effect may be related to an overall lower potency to impair innate mediator production during pathogenic infection. Our data are supported by findings from a recent trial showing that addition of beclomethasone did not increase pneumonia frequency compared with dual bronchodilator therapy alone.

Some clinical studies have reported that budesonide has lesser effects on pneumonia risk than FP, with in vitro studies suggesting that this may be related to differential effects on bacterial adhesion or receptor expression. We observed no difference in the ability of FP and budesonide to impair cathelicidin and increase bacterial burden. In contrast to these other studies, which have solely used in vitro experiments, we report equivalent effects of FP and budesonide both in vitro and in vivo. Unlike some studies, we did not attempt to adjust for dose equivalence between the three ICS in our experimental models, because it is extremely difficult to accurately recapitulate differences in effective doses/complexities of inhaled human drug administration between individual ICS agents in which pharmacokinetics may be affected by several factors, including inhaler device, particle size, aqueous solubility, and epithelial permeability. We instead examined a full concentration range for all three agents to capture the full spectrum of doses and, in vitro, show that a 100-fold higher dose of BDP (which far exceeds the reported approximately 2:1 equivalence ratio) still failed to have comparable effects to a 1-nM dose of FP or budesonide, thus clearly showing a lesser potential for BDP to impair antibacterial immunity. Our data therefore indicate that BDP potentially has lesser beneficial antiinflammatory effects but conversely has reduced potential to impart the detrimental effect of inhibiting protective antimicrobial responses.

We have previously reported that effects of FP on cathelicidin and bacterial replication occur consistently in healthy or diseased (COPD) models. Our studies here were conducted in nondiseased airway epithelial cells and mouse infection models. Whether similar differences between ICS agents occur in smoke exposure animal models or primary cells from patients with COPD, the group at greatest risk of developing ICS-related pneumonia, remains to be seen. COPD patients are most commonly treated with ICS combined with long-acting bronchodilators; future studies should evaluate effects of clinically relevant combination therapies and also assess other bacterial pathogens of importance in COPD such as Haemophilus influenzae.

Head-to-head clinical trials of different ICS agents examining effects on immune mediators, microbiota, and pneumonia development will ultimately be required to confirm that these findings are relevant to human disease.

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