

Bronchial Hyperreactivity Related to Inhalation Therapy in Cystic Fibrosis Patients

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Abstract

It is presumed that bronchial hyperreactivity (BHR) can occur with any inhaled agent and may be a reason for discontinuation of inhalation therapy in cystic fibrosis (CF) patients. On the other hand, inhalation of antibiotics is being increasingly used to eradicate or treat infections.

This review focuses on identifying the mechanisms of BHR for a better understanding of its impact on inhalation treatment. BHR in CF is suggested to be secondary to underlying airway disease (associated to poor pulmonary function, chronic inflammation and aerosol distribution) or to be a separate condition occurring more in CF. Furthermore, certain characteristics of the aerosol solution itself, such as the active molecule, chemical additives and particle size, can cause BHR.

Recombinant human DNase (rhDNase), hypertonic saline (HS) and the antibiotics tobramycin, colistin and aztreonam lysine for inhalation (AZLI) are frequently used inhalation drugs in the treatment of CF. Prevalence of BHR related to both short and long-term inhalation of these drugs as reported in the literature was investigated. Acute BHR is documented in up to two thirds of CF patients. Despite the widespread use of rhDNase, HS, tobramycin, colistin and AZLI, only one long-term trial looked for, but did not demonstrate, BHR at the end of the trial period.

Keywords: Cystic fibrosis; Bronchial hyperreactivity; Inhalation therapy; Recombinant human DNase; Hypertonic saline; Tobramycin; Colistin, aztreonam lysine for inhalation solution

Introduction

Cystic fibrosis (CF) is the most common life-shortening autosomal recessive multi-organ disease; it is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [1,2]. The function of CFTR is important in many organs, including sinuses, airways, pancreas, liver, and the reproductive tract. However, the most important site of disease and the predominant cause of both morbidity and mortality in CF is the respiratory tract [3,4]. CFTR deficiency results in a modified epithelial ion transport and alters airway epithelial cell function by changing epithelial surfaces, leading to increased bacterial adherence and exaggerated airway cell inflammatory responses. These modifications lead to changes in both quality and quantity of mucus secretions and eventually lead to impairment of mucociliary clearance and loss of pulmonary function [5].

In the past 25 years, several factors relating to preservation of pulmonary function have been implemented in the standard of care among CF patients, focusing on two main aspects [6,7]. The first aspect is the need to keep the airways clear of mucus secretions using

physiotherapy, nebulized recombinant human DNase (rhDNase) and/or hypertonic saline (HS). rhDNase in particular has been widely used since the mid-1990s and has participated to achieve an improved airway clearance, improved pulmonary function, fewer exacerbations and, most importantly, an improved survival [6]. The second aspect is the suppression of bacterial colonization and infection with intravenous and/or inhaled antibiotics, [6] such as tobramycin, colistin and aztreonam lysine for inhalation (AZLI).

Airway reactivity, hyperreactivity, and hyperresponsiveness are terms used to describe an increased bronchial smooth-muscle tone or responsiveness of the airways [8]. This is manifested (1) by airway obstruction on a pulmonary function test and is acutely responsive to a bronchodilator, or (2) by airways that respond transiently with bronchospasm to various stimuli, such as inhaled agents. Bronchospasm may occur with any inhaled agent, [9,10] but the risk of occurrence is higher with the use of solutions containing preservatives or with a significantly different osmolality compared to that of the airway secretions [9-11].

The aims of this review are (1) to identify the mechanisms of bronchial hyperreactivity (BHR) and (2) to investigate the prevalence of BHR, as reported in the literature, related to both short and long-term inhalation of five regularly used wet inhalation drugs in CF

treatment, i.e. rhDNase, HS, and the antibiotics tobramycin, colistin and AZLI.

Methods

Search methods for studies

Full papers in the English language were selected from the PubMed, Web of Science and CEBAM databases (latest review October 2014). Keywords (MeSH terms) (+ combinations) were: cystic fibrosis, bronchial hyperreactivity, bronchial spasm, inhalation, dornase alfa, rhDNase, hypertonic saline solution, tobramycin, colistin and aztreonam lysine for inhalation.

Criteria for considering articles

Type of studies / interventions / participants

Randomized or quasi-randomized clinical trials and reviews on the use of inhaled rhDNase, HS, tobramycin, colistin and aztreonam lysine for inhalation delivered by any device for wet nebulization at any frequency and for any duration in all patients with diagnosed CF.

All types of articles on bronchial hyperreactivity in CF.

Type of outcome measures

Documented BHR defined as increased wheezing, cough, dyspnea, chest tightness and/or airflow obstruction on pulmonary function test.

Evidence levels

Cited evidence levels are based on the Oxford Centre for Evidence Based Medicine levels of evidence.

Bronchial Hyperreactivity

Assessment of BHR

Bronchial hyperreactivity (BHR) is defined as an abnormal increase in airflow limitation following exposure to a certain stimulus and is usually accompanied by airway obstruction, which is visible on a pulmonary function test [12,13]. As BHR can be observed with any inhaled agent, many agents can be used for bronchoprovocation challenge tests (BCTs) [9,10,14]. Whilst it is beyond the purpose of this review to describe all BCTs, Figure 1 gives a general overview of how these different tests can be categorized.

Numerous methods, of which metacholine or histamine inhalation are the most commonly used, have been applied to assess BHR and end points vary between a 10 to 20% fall in forced expiratory volume in one second (FEV1) depending on the chosen BCT protocol. Responsiveness is then reported as the provocation concentration (PC) or provocation dose (PD) causing the targeted fall (10-20% depending on the protocol of the trial) in FEV1 (PC10-20 or PD10-20) [12-15]. A bronchodilator is always administered at the end of a BCT [12].

For detailed description of the BCT protocols, the authors would like to refer the interested reader to the comprehensive reviews by Sterk et al., Joos et al., Cockcroft et al. and Anderson et al. [12-15].

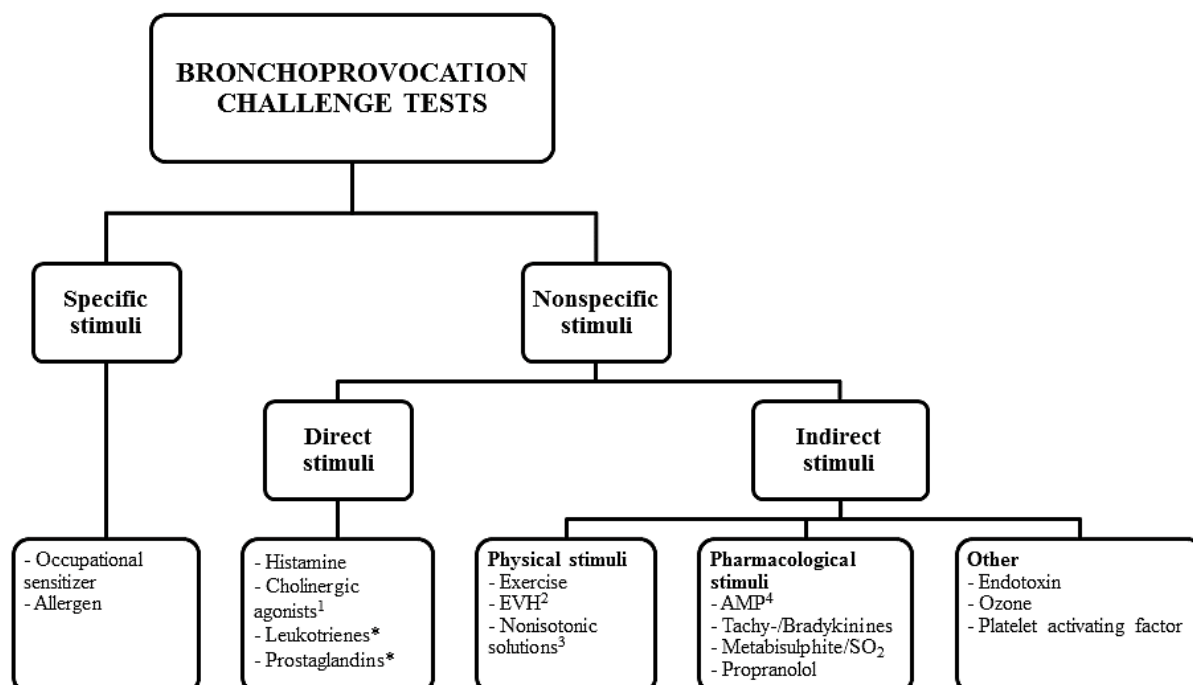


Figure 1: Categorization of bronchoprovocation challenge tests. 1Cholinergic agonists such as metacholine, acetylcholine; 2Eucapnic voluntary hyperpnoea of dry air; 3Nonisotonic solutions such as hypertonic saline, distilled water, mannitol; 4Adenosine 5'-monophosphate; *For research purposes only.

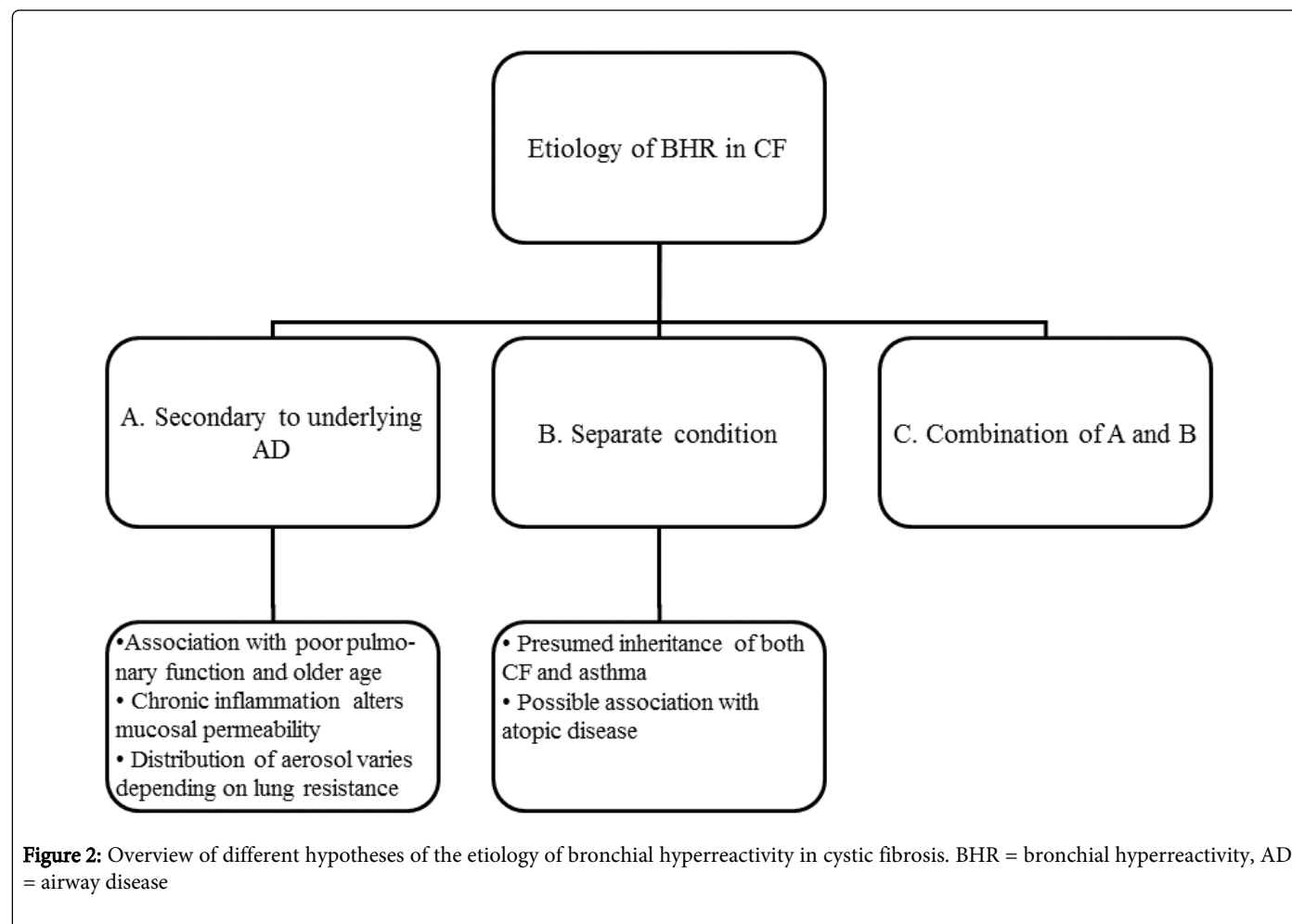
Mechanisms of BHR

Many CF patients demonstrate BHR, characterized by airway obstruction, dyspnea, wheezing and coughing [16]. Airway obstruction in CF involves several overlapping mechanisms, including (1) mechanical obstruction by excessive viscous secretions, (2) airway mucosal edema secondary to chronic infection and inflammation, (3) increased compressibility of airways during expiration because of the destruction of airway walls, and (4) airway smooth muscle contraction due to stimulation of autonomic nerve fibers caused by damage to respiratory epithelium [11,16-18].

Bronchospasm may occur spontaneously or be triggered by any inhaled agent [9,10]. Estimates are that about half of the CF patients demonstrate significant airway narrowing upon administration of these pharmacological agents [8,11,17,19-26]. According to Weinberger et al., cohort studies support the presence of two types of airway reactivity in CF: (1) CF patients with concomitant clinical asthma who have BHR in response to metacholine, histamine and exercise; and (2) BHR in CF patients without concomitant clinical

asthma [8,19,22,24,25]. The latter have BHR which differs from what is seen in asthma. For example, Mitchell et al. demonstrated BHR to metacholine in 51% of CF patients; only 21% of these patients had BHR to histamine as well. However, all histamine-responders responded also to metacholine. In asthma patients, no differences were detected in reaction to metacholine and to histamine [19]. In addition, Van Asperen et al. reported histamine reactivity to be blocked by an anticholinergic aerosol only in patients without coexistent asthma [24].

Although BHR is a functional characteristic of asthma, the etiology of BHR in CF is considered multifactorial and different hypotheses about the etiology of BHR in CF have been proposed (Figure 2) [8,16-19,21,23-25]. Some authors suggest BHR occurs secondary to underlying airway disease in CF [16,20,21] others suggest it is a separate condition that occurs more commonly in CF, [8,22] while a third group believes BHR is the result of both mechanisms [8,19,23,24].



Three main explanations have been suggested to support the hypothesis of BHR occurring secondary to underlying airway disease, i.e. association with poor pulmonary function and age, chronic inflammation and changes in aerosol distribution. First, some authors found a strong association between a positive metacholine or histamine challenge test and poor pulmonary function (FEV1 less than

40% predicted [26,27]) [16,19,21,22]. Underlying airway narrowing and thickening may alter the airway geometry so that a small degree of narrowing produces an important relative change in cross-sectional area [16,21]. On the other hand other authors found no difference in FEV1 in reactive patients compared to non-responders [23,28]. Furthermore, an association between a positive response and older age

was observed by Mellis et al., [21] while others could not confirm this finding [19,23]. This means BHR may become only present over time as patients get older and/or progress to more severe illness. These findings are similar to what is seen in the general population. Scichilone et al. described an overall positive association between age and BHR, with an increased prevalence in the elderly [29]. Second, chronic inflammation in CF may lead to the development of BHR by altering mucosal permeability, thus allowing a better penetration of inhaled agents into bronchial smooth muscle and on to irritant neural receptors [16,19-21,23]. This conclusion is consistent with the observation that BHR to histamine is increased in non-CF subjects during, and for several weeks after a viral upper respiratory tract infection. Presumably, viruses cause airway epithelial damage, altering the responsiveness of the irritable neural receptors, leading to an exaggerated response to histamine [21]. Similarly, early-life infection with respiratory syncytial virus in the general population is suggested to be one of the most important risk factors for the development of childhood asthma by deregulating local immunity [30]. In addition, Van Haren et al. demonstrated in a placebo-controlled trial that treatment with inhaled corticosteroids decreases BHR in adult patients with CF, supporting the hypothesis that chronic inflammation can lead to the development of BHR [25]. Third, the distribution of inhaled aerosol throughout the respiratory tract varies depending on particle size, inspiratory flow rate, and total lung resistance [16]. High airway resistance in the CF patient with advanced airway disease will presumably alter the distribution of the aerosol, favoring increased deposition in central rather than in peripheral airways. Selective deposition of histamine, or any other inhaled agent, in central airways could cause more pronounced changes in the large airways than if it were more evenly distributed [16,19,21]. Additionally, particles larger than 5µm in diameter cause cough as a result of inertial impaction at the level of the posterior pharynx. As particle size is directly related to adverse effects, the type of nebulizer used can affect the incidence and/or severity of observed airway obstruction [31].

The association between BHR and CF has also been attributed to concomitant defects, in particular to the presumed inheritance of both CF and of asthma. CF patients with a normal pulmonary function (FEV1 >90% predicted [26]) and a clinical history of asthma were generally more sensitive to inhaled histamine compared to those with no history of asthma [23]. Some authors describe a possible association with atopic disease [23,24]. CF histamine-responders had a higher incidence of wheezing and infantile eczema compared to non-responders, although there was no difference in the frequency of family history of asthma [23]. On the other hand, other authors found no correlation between BHR and atopic status [19,28]. Van Asperen et al. suggest that children with CF who react to typical asthmatic allergens are those with genetically determined atopy; thus supporting the hypothesis that BHR can reflect underlying asthma [23]. However, the reported incidence of BHR of 40% in mild CF patients [23] (FEV1 70-89% predicted [26,27]) is higher than the generally accepted figure of 10-25% in the general population, [8,23] suggesting coexisting asthma is not the only cause of histamine-induced BHR in CF. CF patients with coexistent asthma are generally older, have lower baseline FEV1 values, are more sensitive to histamine, are more atopic, and are often not successfully protected from histamine challenge by a bronchodilator [24]. Whether these characteristics relate to a true asthma tendency or simply reflect more advanced CF airway disease is unclear [18].

The occurrence of bronchial narrowing during or after inhalation of a certain solution might also be associated with the formulation of the

aerosol solution itself, e.g. the active molecule and chemical additives [32]. The osmolality of the solution appears to be an important factor in how the nebulization is tolerated by the patient [9,33,34]. Both hypo- and hypertonic solutions may result in mucosal irritation [9-11,32,33]. In addition, a number of chemical additives in the nebulizing solution, e.g. antioxidants and preservatives, can cause airway narrowing [32,35].

In summary, BHR in CF is hypothesized to be either secondary to underlying airway disease (association with poor pulmonary function, chronic inflammation and altered aerosol distribution), either a separate condition that occurs more in CF (presumed inheritance of asthma and possible association with atopic disease), either a combination of both hypotheses. Furthermore, certain characteristics of the aerosol solution itself can cause BHR.

Despite the controversial knowledge about the mechanisms of BHR in CF, inhaled bronchodilators are frequently prescribed [8,16,17,20]. Bronchodilator therapy may increase mucociliary transport, decrease inflammatory damage to the airways, increase exercise tolerance and decrease dyspnea [8,36,37]. Response to short-acting inhaled bronchodilators is variable over time and between patients; [8,17,19,22,24] approximately 50 to 60% of the patients show an improvement of up to 15% in forced expiratory volume in one second (FEV1), 30% of patients show no change and a further 10 to 20% deteriorate with lower FEV1 values after inhalation of a bronchodilator [17]. A Cochrane review concluded that both short and long acting beta-sympathomimetics can be beneficial for CF patients who experience BHR and respond to bronchodilator inhalation [37].

Prevalence of BHR

Prevalence of BHR related to the inhalation of the mucolytics rhDNase and HS and the antibiotics tobramycin, colistin and AZLI, as discussed in the upcoming sections, varies between 2 and 66%, depending on the type of drug, its intrinsic characteristics and different measurement of BHR in the different trials (Table 1).

Drug	Reported prevalence of BHR
rhDNase	0-35%
Hypertonic saline	3-30%
Tobramycin	
Preservative-containing solution	19-66%
Preservative-free solution	8-42%
Colistin	11-37.5%
AZLI	2-8%

Table 1: Prevalence of BHR as reported in the literature

The current review is restricted to the wet inhalation of these drugs because their use in (European) CF patients is well established. However, it should be noted that dry powder formulas of both mucolytics, e.g. mannitol, and antibiotics, e.g. tobramycin, recently gained more interest and are briefly discussed in the last section ('DPIs'). For each eligible study, the type of study and reported data on duration, sample size, dose/concentration, (pre-)treatment with a bronchodilator or inhaled corticosteroids were recorded

(Supplementary Tables 1-5), as well as the prevalence on BHR, dyspnea, wheeze, cough and reduction in FEV1 of (more than) 10 to 20% (depending on the trial's targeted end point). Key publications investigating BHR related to inhalation of different drugs are listed in Table 2.

BHR and mucolytics

The airways of CF patients are characterized by the production of large amounts of thick, tenacious secretions, which are cleared ineffectively by the usual mucociliary escalator [38]. Chronic infection in CF leads to the migration of large numbers of neutrophils into the airways. Here the neutrophils eventually degenerate and disgorge their cellular contents, [38] including large quantities of nuclear DNA and cytoskeletal actin, both of which are known to markedly increase the viscosity of sputum when released into the airway [1]. Hence, inhalation of mucolytics and clearance of viscous airway secretions with physiotherapy are a cornerstone in the management of established CF pulmonary disease [39-41]. The most common mucoactive agents used in CF care are rhDNase and HS [41].

Drug	[Reference] Author, Year
rhDNase	[45] Eisenberg JD et al., 1997 [50] Milla SE et al., 1998
Hypertonic saline	[11] Rodwell LT et al., 1996 [68] Elkins MR et al., 2006 [67] Rosenfeld M et al., 2011
Tobramycin	[84] Nikolaizik WH et al., 1996 [77] Ramsey BW et al., 1999 [32] Pai VB et al., 2001 [35] Althman GA et al., 2002
Colistin	[92] Maddison J et al., 1994 [94] Cunningham S et al., 2001 [95] Hodson ME et al., 2002 [10] Althman GA et al., 2005
AZLI	[109] Retsch-Bogart GZ et al., 2008 [110] McCoy KS et al., 2008 [111] Retsch-Bogart GZ et al., 2009 [113] Oermann CM et al., 2010

Table 2: Key publications investigating BHR related to inhalation of rhDNase, hypertonic saline, tobramycin, colistin and AZLI

rhDNase

Since the human enzyme DNase I was cloned and sequenced in 1990, [42] rhDNase has become the most widely used treatment to reduce sputum viscosity [5,43] rhDNase digests polymeric extracellular DNA, thereby reducing the viscoelasticity of CF sputum and increasing mucociliary transport [36,42]. Long-term use of rhDNase reduces the frequency of pulmonary exacerbations and delays decline in pulmonary function [6,44,45].

BHR has been observed in one trial investigating intermittent inhalation (14 days on/off) of rhDNase during 24 weeks (evidence level 1B). Sixty-five out of 184 (35%) patients experienced 106 episodes of airway reactivity in response to nebulized rhDNase [45]. However,

most studies did not demonstrate significantly more BHR, dyspnea, cough, wheeze and/or increased pulmonary obstruction in CF patients compared to controls (Supplementary Tables 1) [42,46-54].

Hypertonic saline

As a consequence of CFTR dysfunction, unrestrained sodium absorption, accompanied by increased restoration of water, and failure of active chloride secretion in the CF airway leads to a depletion of airway surface liquid volume, subsequent abnormal mucociliary clearance and promotes the formation of adherent mucus plaques on airway surfaces [1,5]. Sufficient high-quality evidence (evidence level 1A) has been collected to recommend inhaled HS as an alternative mucolytic agent in CF treatment [55]. HS has a favorable effect on mucus rheology in vitro through hydration of the airway surface [40]. Another postulated effect of HS is that by increasing salt concentrations on the luminal side of the respiratory epithelium the viscous mucus will hydrate, improving mucociliary clearance [41,56-61]. The landmark trial by Elkins et al. (evidence level 1B) demonstrated that 48-weeks treatment with 7% HS nebulized twice daily improved the absolute difference in pulmonary function between groups, but not the decline in pulmonary function over time. Furthermore, it reduced the frequency of pulmonary exacerbations in both children older than 6 years and adults [62]. However, the ISIS trial (evidence level 1B), performed in children with CF aged 4 to 60 months, could not demonstrate the same effects of HS in this specific age group [63].

Nebulized HS can induce acute airway narrowing or bronchospasm and cough (Supplementary Tables 2), [11,55-69] but in most cases these symptoms decrease after a few weeks so that the majority of patients find HS tolerable [55,62,63,68]. Rodwell et al. were the first to describe acute airway responsiveness to inhaled 10% saline in a group of CF subjects with asthma-like symptoms [11]. Approximately 30% of the subjects responded to the inhaled 10% saline by progressive and persistent airway narrowing as observed in asthma. The remainder of the subjects (resp. 39% and 30% of the patients) experienced transient airway narrowing with nearly full recovery or even an improvement (+4.5%) in pulmonary function by the end of the NaCl 10% challenge test. Most subjects in all 3 groups had an improvement in pulmonary function after administration of a bronchodilator at the completion of the challenge [11].

Although excessive coughing can be an unpleasant adverse effect, the tussive effect of HS is an important mechanism to improve mucociliary clearance [57,58,68,69]. A meta-analysis of studies examining regular HS inhalations in patients with CF indicated that intolerance due to cough occurs in 3% and due to any symptom in 8% of patients [68].

To our knowledge no study investigated newly developed BHR after inhalation of HS for more than six months. Only one study reported on new development of BHR to HS in one participant at the end of a 2-weeks trial [67]. However, Elkins et al. found cough to resolve after a mean of 15 days [62].

BHR and nebulized antibiotics

The airway is continuously challenged by inhaled pathogens, which, when inhaled in small quantities, are usually cleared without provoking significant inflammation. However, CF patients are particularly vulnerable to respiratory infections [3] Dense plaques of mucus become adherent to the epithelial surface from which bacteria

cannot be cleared normally [1,3]. While *Haemophilus influenzae* and *Staphylococcus aureus* may predominate early in life, approximately 15-30% of children with CF 2-5 years of age and 65-80% of young adults aged 25-34 years are chronically infected with *Pseudomonas aeruginosa* (Pa) [3,36,70-72]. The association between chronic colonization with Pa and worsening pulmonary function is well known [7]. Respiratory infections with Pa are difficult to treat, e.g. due to growth of the pathogen in biofilm-like macro-colonies and multiresistance of Pa [72,73]. Nevertheless, various treatment strategies have been developed and have a significant positive impact on prognosis of Pa colonization. To avoid adverse effects and to obtain high airway concentrations, the inhalation route has been increasingly used in CF patients [4,9,36,73-75]. The benefits of nebulized antibiotics in CF consist of a reduction in pulmonary function decline and in the number of hospital admissions for patients with severe disease, a reduction in positive sputum cultures in patients recently colonized with Pa, and eradication of the organism [34,76,77].

Since the early 1980s, several trials have studied inhaled antibiotics as a suppressive treatment of chronic Pa infection in CF [10,31-35,76-103]. As no approved formulations for inhalation were available, drugs were composed from intravenous formulations containing preservatives, with doses selected not by preclinical safety or efficacy testing, but based on how parenteral formulations were composed [75]. Different studies demonstrated airway narrowing and chest tightness after inhalation of these nebulized antibiotics [33,97,98]. In Europe, nebulized tobramycin and colistin are used after initial isolation of Pa, to treat acute infection and to delay and/or treat chronic infection [44,97,99-101]. These two antibiotics, belonging to the antibiotic classes of aminoglycosides and polymyxins respectively, have been for more than 20 years the only ones to be used routinely and eventually to be approved for inhalation in CF patients with Pa infection. More recently, existing antibiotics for intravenous use were adapted to be suitable for inhalation, mostly by lyophilisation, of which the beta lactam antibiotic AZLI is EMA and FDA approved for anti-Pa therapy since 2009 and 2010, respectively. Other of these adapted antibiotics, such as amikacin and levofloxacin, are still under investigation in clinical trials. Moreover, short courses of off-label inhalation of antibiotics for intravenous use, e.g. meropenem, ceftazidime, carbenicillin and amphotericin, are reported in small series of CF patients [26,104-106]. However, data on the relation with BHR upon inhalation of these antibiotics are scarce.

This review will only focus on tobramycin, colistin and AZLI, all three bactericidal drugs active against Pa, and well established in CF for use via inhalation [4,36,105]. These drugs have initially all been developed and studied as monotherapies.

Tobramycin

Nebulized tobramycin has been the most thoroughly studied chronic suppressive antibiotic therapy [27]. It is mainly used as a maintenance therapy in patients chronically infected with Pa to slow the progression of airway disease and to increase the interval between exacerbations. Two main preparations of inhaled tobramycin have been studied in clinical trials; a preservative-free formulation (TIS) with a concentration of 60mg/ml developed for inhalation purposes solely, and intravenous forms with concentrations ranging from 20 to 50mg/ml which contain preservatives and antioxidants, and are diluted with saline [35].

Short-term adverse effects of airway narrowing have been observed after inhalation of both the preservative-containing solution (19-66%

of patients) [32,76,81,84] and the preservative-free solution (8-42% of patients) (Supplementary Tables 3) [35,82,88,94]. Some authors did not report on or did not find airway narrowing, [77-80,83,86-87] or did not specify whether airway narrowing itself was one of the observed 'adverse events' [90]. Airway narrowing within 30 minutes post-inhalation of the preservative-free solution was transient and similar to that observed after inhalation of placebo [77]. However, some authors report up to 10% patient withdrawals due to intolerability of tobramycin inhalation [35,85,101]. This intolerability can appear either immediately [35,85,101] or after a few weeks of tobramycin inhalation [85]. Long-term studies demonstrated inhalation of tobramycin to be tolerable.[7,77,87,95] Ramsey et al. specified that they did not find bronchospasm on the challenge test at the end of a 24-weeks placebo-controlled trial in 520 patients (evidence level 1B) [77].

Colistin

Some patients with advanced airway disease harbor tobramycin-resistant strains of Pa in their respiratory tract. This has prompted the search for other inhalation antibiotics with antimicrobial activity against Pa [10]. Colistin sulphomethate is a polymyxin antibiotic that is preservative-free; it is produced as a dry powder preparation which is reconstituted with saline prior to nebulization [93]. Colistin is generally well tolerated. Nevertheless, acute clinically significant airway narrowing is a quite frequent complication following inhalation of colistin, occurring in approximately 17 to 37.5% of patients (Supplementary Tables 4) [10,34,74,92-94,96]. The reaction occurs almost immediately after initiation of inhalation and can extend beyond 30 minutes in some individuals [92,94]. A Cochrane review on long-term inhaled antibiotic therapy identified two placebo-controlled trials on the efficacy of inhaled colistin [102]. The trial with the longest duration (6 months) presents limited detail in the abstract, which is the only form of publication [102]. The 3-months study by Jensen et al. did not demonstrate colistin to cause bronchospasm [91]. When compared to nebulized tobramycin, 4-weeks inhalation of colistin was associated with fewer reported drug-related adverse effects (50% vs 64%). However, a greater number of patients receiving colistin exhibited bronchospasm (17.7 vs 11.3%) (evidence level 1B) [95].

Aztreonam lysine for inhalation (AZLI)

Another antibiotic that is developed for patients who harbor tobramycin-resistant strains of Pa, is AZLI. Aztreonam has been used via the parenteral route to treat infections caused by aerobic gram-negative bacteria, including Pa, and was reformulated as a lysine salt for inhalation delivery [105]. Randomized, double-blind placebo controlled trials [108-112] and open label studies [113,114] investigated the safety profile of AZLI (Supplementary Tables 5).

Bronchial hyperreactivity, defined as a decrease of > 15% in FEV1, was observed in four short-term trials (28 days) with prevalence ranging from 2-8% [108-111]. Retsch-Bogart et al. found that after one week of therapy, BHR following AZLI inhalation was not different among groups [109]. The amelioration of BHR seen after one week of AZLI administration was attributed to the fact that (1) patients became accustomed to the drug / delivery system and to (2) the bacterial killing by AZLI and the subsequent reduced inflammatory stimuli which may in turn have reduced BHR. Only Oermann et al. reported withdrawal of ten patients due to study drug intolerance, the other trials did not report any withdrawal for this reason [113].

Causality of BHR related to antibiotics

Several explanations for airway narrowing in reaction to nebulized solutions of tobramycin, colistin or AZLI have been cited by some authors, but questioned by others (Table 3). Patients receiving hypertonic antibiotic therapy have been shown to have more airway narrowing compared to patients receiving antibiotics with lower tonicity, [10,81,102] although this effect might not always be very pronounced [34,35,88]. For example, Dodd et al. found no effect of

solution tonicity on the occurrence of BHR related to inhalation of colistin, but they did demonstrate an effect on time to maximum fall in FEV1 [34]. Fall in FEV1 occurred earlier after inhalation of a hypertonic colistin solution compared to inhalation of a hypo- or isotonic colistin solution. Some patients may tolerate a gradual fall in FEV1 over a longer time period better than an equivalent abrupt fall such as occurred with the hypertonic colistin solution [34].

Reason	Tobramycin		Colistin		AZLI	
	Yes	No	Yes	No	Yes	No
Coexisting tendency for BHR	35,84	81,88	10,92	-	-	-
Tonicity of the solution	35,81,88	35,88	10,103	34	-	-
Concentration/dose of the solution	88	35,84	-	-	109,110	-
Active molecule itself	-	-	10	-	-	-
Presence of preservatives	35,77,82,84	-	-	-	-	-

Table 3: Possible reasons for BHR related to the inhalation of nebulized tobramycin and colistin (presented as citing reference)

DPIs of Mannitol, Tobramycin and Colistin

Mannitol

Mannitol is a sugar alcohol, which, when inhaled, creates an osmotic gradient that is thought to facilitate movement of water into the lumen of the airways. This will increase the volume of the airway surface liquid and improves mucociliary clearance [115-117]. To date, two long-term trials (26 weeks) investigated the efficacy and safety of inhaled dry powder mannitol in CF patients [115,116] and a third long-term trial is running (NCT02134353).

Although mannitol is currently used as stimulus in bronchial provocation tests (Figure 1), and thus may cause bronchoconstriction, BHR was reported in only two patients by Bilton et al. [115] and was not observed by Aitken et al. [116].

Tobramycin and colistin

Treatment of Pa infection by inhalation of wet antibiotics is time consuming and places a high burden on CF patients [118]. Therefore, dry powder inhalation formulations (DPIs) were developed during the last decade for the delivery of both tobramycin and colistin [118-122]. Konstan et al. investigated safety and efficacy of tobramycin inhalation powder (TIP) vs. placebo and vs. TIS [118,119]. Both 24-week trials demonstrated TIP to be safe as AEs were similar in both groups. Decrease in FEV1 of more than 20% was similar in TIP vs. TIS (5.2% vs. 5.3%) and TIP vs. placebo (0.05% vs. 0.08%) [118,119]. However, incidence of cough was higher in the TIP vs. TIS-treated patients (48.4% vs. 31.1%; $p < 0.001$) [118].

Safety and efficacy of colistin dry powder for inhalation (CDPI) compared to TIS was investigated in a 24-week trial by Schuster et al. [122]. Number of AEs was equal for both groups, except for cough which was higher in patients receiving CDPI (75.4% vs. 43.5%).

Increased cough due to DPI therapy is typical, as inhalation of these products causes throat irritation and unpleasant taste due to oropharyngeal deposition. Another possible explanation of increased

cough is the relatively higher drug payload of TIP and CDPI [118,122]. Children and adolescents tended to cough more with TIP, whereas adults experienced more cough with TIS [118]. Further follow-up of these drugs and objective assessment of BHR on longer term, i.e. >26 weeks, is required.

Conclusion

Approximately half of the CF patients experience bronchial hyperreactivity on a metacholine challenge test, but not all of these patients also respond with BHR to histamine as seen in asthma. This demonstrates that these challenge tests are not specific enough to look for BHR in CF patients. BHR in CF is suggested to be secondary to underlying airway disease (associated to poor pulmonary function and age, chronic inflammation and altered aerosol distribution) and/or to be a separate condition that occurs more in CF. BHR may be a reason for poor compliance and mandatory discontinuation of inhalation treatment, and is therefore an important side effect which needs full attention because it may narrow the therapeutic possibilities. New developments in inhalation therapy replacing wet aerosols by dry powder inhalation, e.g. mannitol, TIP and CDPI, as well as the inhalation of antibiotics for intravenous use for the suppression of difficult to treat respiratory pathogens, require close follow-up regarding to the development of BHR.

Acute BHR is reported after inhalation of rhDNase, HS, tobramycin, colistin and AZLI with different prevalence (2-66%). Therefore, tolerability of these inhalation drugs should always be tested at the beginning of a new treatment. If patients are found to be intolerant to the tested drug, the use of beta-agonists could be recommended as they may prevent, though not in all cases, the occurrence of BHR upon inhalation of these drugs. Furthermore, it might be considered to retest the tolerability when the CF patient is in a stable period, to reduce the influence of underlying airway disease to a minimum.

Despite the widespread use of rhDNase, HS, tobramycin, colistin and AZLI, little is known about the prevalence of BHR as a result to

chronic inhalation of these different drugs. Long-term trials should assess BHR not only at the start, but also at different follow-up intervals and at the end of the trial period.

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