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Research paper

Mechanistic appraisal of the effects of some protease inhibitors on ciliary beat frequency in a sequential cell culture system of human nasal epithelium

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Abstract

The aim of this study was to investigate the suitability of a sequential monolayer-suspension culture system as a model to screen subacute effects of drug excipients on ciliary beat frequency (CBF). The CBF of the cultured cells was measured by computerized microscope photometry. Protease inhibitors (puromycin, bestatin, bacitracin, actinonin and thiomersal) were used as model compounds and the mechanisms of ciliary inhibition were investigated by probing the involvement of arachidonic acid metabolism, guanylate cyclase (cGMP), protein kinase C (PKC) and adenosinetriphosphate (ATP) inhibition. Bestatin concentration-dependently reduced CBF by inhibiting arachidonic acid metabolism, cGMP, PKC and endogenous ATP consumption. Thiomersal and DMSO used for dissolving actinonin reduced CBF ($P < 0.05$) via a non-specific mechanism. Bacitracin (8 mM) and puromycin (135 mM) had no effect on CBF after acute exposure (15–30 min) ($P > 0.05$), but significantly reduced the CBF by approximately 15.0% following daily15-min exposure for 1 week. This study shows that (i) sequential monolayer-suspension culture system is a valid model to screen both acute and subacute effects of drug excipients on CBF; and (ii) bacitracin, puromycin and actinonin are more cilio-compatible than bestatin and thiomersal and as such are more potentially useful nasal absorption enhancer from ciliotoxicity perspective.

Keywords: Nasal drug delivery; Cell culture; Ciliary beat frequency; Ciliotoxicity; Protease inhibitors

1. Introduction

Absorption enhancement and toxicity are the key issues in the search and design of effective and safe drug formulations for the nasal route. Both absorption enhancers and protease inhibitors have been used to promote nasal absorption of proteins and peptides [1]. Some of these excipients impair the mucociliary clearance system [2] and ultimately result in recurrent or chronic infections. Unlike most absorption enhancers used for nasal drug delivery, there is no documented information on the effects of protease inhibitors on human mucociliary transport system or ciliary beat frequency.

Several experimental approaches based on both human and animal tissues have been developed to study the acute effects of pharmaceutical compounds on ciliary activity in vitro [3]. Given the fact that most nasal drug formulations are given on a subacute or chronic basis [4], it is pertinent to have an accurate and reproducible in vitro model for rapid assessment of potential subacute and chronic deleterious effects of pharmaceutical formulations on ciliary beat frequency (CBF).

It has been shown that sequential monolayer-suspension culture system of human nasal epithelium (with ciliated and mucin producing cells) can be maintained in culture with retention of cell viability and physiologically relevant CBF for up to 6 months [5]. This cell culture system has been reported to be an efficient model for (a) medical diagnosis of primary and secondary ciliary kinesia; (b) assessment of acute effects of pharmaceutical compounds on ciliary beat frequency (CBF); (c) molecular and biochemical characterization of centriolar and/or ciliary components (markers of

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ciliated cell differentiation); and (d) assessment of MUC5AC gene expression (marker of mucin producing cell differentiation [6–10]. Based on these studies, the sequential monolayer-suspension culture system seems to be a promising model for in vitro studies on the subacute or chronic effects of pharmaceutical compounds on CBF.

The aim of this study was to apply the sequential monolayer-suspension culture system as a model to investigate both acute and subacute effects of protease inhibitors (puromycin, bestatin, bacitracin, actinonin and thiomersal) on human ciliary activity and to elucidate the possible mechanisms of ciliary inhibition. For assessment of the possible mechanisms of ciliary inhibition, the involvement of arachidonic acid metabolism, guanylate cyclase (cGMP), protein kinase C (PKC) and adenosinetriphosphate (ATP) were investigated.

2. Materials and methods

2.1. Chemicals

The chemicals used to culture the cells (streptomycin, penicillin, physiological saline, DMEM-F12 1/1, phenol red, collagenase, pronase, Ultroser G, NU-serum), puromycin and bestatin were procured from Life Technologies (Paisley, UK). Bacitracin and dimethylsulfoxide were obtained from Fluka (Buchs, Germany) and BDH (England, UK), respectively. Actinonin, methylene blue, H-7 ([1-(5-isoquinolinesulfonyl)-2-methyl-piperazine]), thiomersal, adenosine-5′-triphosphate disodium salt hydrate (5′-ATP-Na_2), pronase and choleratoxin were supplied by Sigma (St Louis, MO, USA). Indomethacin and methylene blue were purchased from Certa (Braine-l′ Alleud, Belgium) and RPL (Leuven, Belgium), respectively.

2.2. Cell culture procedure

The cells were cultured as described previously by Jorissen et al. (1989. Human nasal epithelial tissues harvested during elective surgery from 11 patients were washed three times in physiological saline supplemented with 50 μg/ml streptomycin and 50 IU/ml penicillin (Boehringer Mannheim, GmbH, Germany). Subsequently, the cells were dissociated enzymatically overnight under continuous rotation at 4 °C using 0.1% pronase in DMEM-F12 1/1. Deactivation of pronase was achieved using 10% NU-serum before washing the cells with the monolayer culture medium [DMEM-F12 1/1 supplemented with 2% Ultroser G, 10 ng/ml choleratoxin, 50 μg/ml streptomycin and 50 IU/ml penicillin]. The cells were washed three times with the monolayer medium before recovery by centrifugation at 70 × *g* for 5 min. Seeding the cells on plastic for 1 h at 37 °C and 5% CO_2 reduced the level of fibroblast contamination by selective attachment of fibroblasts to plastic.

Subsequently, cells were plated at a density of 5×10^5 cell/cm^2 in T 75 tissue culture flasks (Falcon, Oxnard, CA, USA) coated with 0.2% collagen gel (extracted from rat tails) and containing 15 ml monolayer culture medium. The medium was changed 1 day after plating and subsequently three times a week. After 3 weeks of cell growth and de-differentiation in the monolayer culture, the cells were released from the collagen gel with 200 IU/ml-collagenase type IV (Worthington Biochemical Corporation, Freehold, NJ, USA). The resulting epithelial clusters of cells were washed three times in the monolayer medium followed by centrifugation at 800 rpm for 5 min and further cultured into several T 25-tissue flasks. In order to avoid cell attachment to plastic, the cells were maintained on a gyrotory shaker at 80 rpm for 1 week. In subsequent weeks, the medium consisted of DMEM-F12 1/1 supplemented with 10% NU-serum. This medium was changed daily during the first week of suspension culture and three times a week after this period. Regeneration of cilia started after 10 days in the suspension culture. Throughout the cell culture duration, the cells were incubated at 37 °C in a 5% CO_2 atmosphere.

2.3. Ciliary beat frequency (CBF) measurements

Three-week-old suspension cultures were used for the acute and subacute studies. The CBF was measured at room temperature (22 °C) using computerized microscope photometry as described earlier [5]. Following incubation for stipulated time intervals, the control (DMEM-F12 1/1) and test solutions (protease inhibitors) were removed leaving the cells adhered to the tissue flask. The CBF of different cells was measured both before and after exposure to the test compounds. Reversibility of cilioinhibition was determined by washing the cells with DMEM-F12 1/1 for 15 and 60 min, respectively after exposure to the test compounds.

The degree of CBF change caused by the test substances and reversibility of the effect was classified as summarized in Table 1 [11,12]. Inhibition studies to assess the mechanisms of cilioinhibition was conducted by pre-treating the cells with indomethacin (3×10^{-6}M), methylene blue (10^{-5} M), H–7 (10^{-5}M) and ATP (10^{-4} M) for 20 min before exposure to bestatin (0.07 mM), thiomersal (0.005%) and DMSO (15.0%) for 15 min.

Subacute studies were also initiated with cells kept in suspension for 21 days and exposure of the cells to the compounds started on day 22, which was considered as day 1 for the subacute study. The mean CBF of the cells at this point in time was 11.9 ± 0.5 Hz. Cells not treated with the test compounds (bacitracin and puromycin) served as a control group on each day the CBF was recorded. The test compounds were exposed to the cells aseptically for 15 min on a daily basis for 7 days. The CBF of the cells were recorded every other day.

Table 1
Classification of inhibitory effects of protease inhibitors on human ciliary beat frequency

Parameters for assessment	Degree of effect	Inference
Onset of cilioinhibition	<10 min	Rapid onset
	10–30 min	Slow onset
	After exposure for 1 week	Delayed onset
Degree of cilioinhibition	<10% or statistically insignificant	No effect
	10–20% cilioinhibition and statistically significant	Mild effect
	20–50% cilioinhibition and statistically significant	Moderate effect
	>50% cilioinhibition and statistically significant	Severe effect
Reversibility of cilioinhibition (%)	>75%	Reversible
	25–75%	Partially reversible
	<25%	Irreversible

2.4. Statistical analysis and data presentation

Unless stated otherwise, the CBF of 20 individual cells was measured (in two batches of 10 cells) before and after exposure to protease inhibitor solutions. The mean CBF change after compound exposure was expressed as percentage ± S.D. For both acute and subacute studies, differences between control and treated cells (before and after compound exposure) were determined by Mann–Whitney *U*-test for unpaired data and Bonferroni's multiple analysis, respectively. The level of significance was set at $P < 0.5$.

3. Results and discussion

In this study, human nasal epithelial cell culture system capable of in vitro ciliogenesis was used to screen the acute and subacute cilioinhibitory effects of bestatin, bacitracin, puromycin, actinonin and thiomersal. The choice of this cell culture model was based on its morphological and functional resemblance with nasal epithelium in vivo, the possibility to keep the cells for a long time with the CBF maintained at physiological range (7–12 Hz), and the results of an earlier validation study [5,6,7,13]. The compounds investigated were selected to represent specific (bestatin, puromycin, actinonin) and non-specific (bacitracin, thiomersal) protease inhibitors and for the fact that most of them have been used to inhibit the nasal proteases [14].

3.1. Acute studies

The results of acute exposures of the inhibitors on the CBF of sequential monolayer-suspension culture system are presented in Table 2. Although lower concentrations of bestain (0.01 and 0.05 mM) had no cilioinhibitory effect, 0.07 mM caused rapid, but reversible CBF reduction by approximately 35% after 15–30 min exposure. While higher concentrations of bestatin (0.1, 0.5 and 1.0 mM) resulted in complete cessation of the ciliary beating, only the effect of 0.1 mM was reversible. It has been shown that bestatin effectively inhibits human nasal aminopeptidases at a concentration of 0.1 mM [15], but a higher concentration (1 mM) is required to bring about nasal absorption enhancement of peptides (e.g. leucine enkephalin) [16]. Nevertheless, the present study shows that bestatin (0.5 mM) causes instant irreversible ciliostatis following exposure to human nasal epithelium in vitro.

Thiomersal, a protease inhibitor, generally considered as a preservative, has not been used as a nasal absorption enhancer for proteins and peptides. It was, however, relevant to see the potential effect of this compound on human ciliary activity. The effect of thiomersal was concentration-dependent and comparable to the effect of bestatin, even though its effect manifested less rapidly and at low concentrations (0.0025–0.01% thiomersal caused 38–70% CBF reduction). The reversibility of the effect of thiomersal was also concentration-dependent with 0.0025–0.01% resulting in less than 50% reversibility and 0.02% in irreversible ciliostasis after a washout period of up to 1 h. The observed ciliotoxcity of thiomersal in the sequential-monolayer-suspension culture system was comparable with the findings of Van den Donk et al. [17]. In their study, it was shown that the irreversible ciliotoxic effect of thiomersal on chicken trachea CBF depended on both concentration and duration of exposure. The demonstrated irreversible ciliotoxicity of bestatin and thiomersal at low concentrations indicate that these excipients may not be useful as nasal absorption enhancers for human use. Under in vivo circumstances, mucus dilution occurs and can possibly attenuate the ciliotoxicity of many compounds. However, this may not be the case with these excipients. For instance 0.1 mM bestatin, which caused irreversible ciliostasis is half of the concentration (0.2 mM) that may result from expected five times mucus dilution of a drug formulation containing 1 mM bestatin under in vivo situation [18].

Actinonin has been found to inhibit enkephalinase A,

Table 2
Results of acute exposures of the inhibitors on the CBF of sequential monolayer-suspension culture system

Compounds	Concentrations	On-set of	Change of CBF		Reversibility after wash-out	
(mM)	inhibition		control		(% relative to control)	
			After 15 min exposure	After 30 min exposure	After 15 min washing	After 60 min washing
Bestatin	0.01	No effect	4.6 ± 0.4 ↑	0.5 ± 7.5 ↓	102.2 ± 1.3	102.2 ± 5.0
	0.05	No effect	6.7 ± 0.2 ↓	4.0 ± 0.5 ↓	101.5 ± 5.5	107.8 ± 7.9
	0.07	Rapid	36.6 ± 6.0 ↓	35.5 ± 4.7 ↓	102.1 ± 3.1	98.1 ± 4.6
	0.1	Rapid	100.0 ± 0.0 ↓	100.0 ± 0.0 ↓	97.5 ± 5.9	97.8 ± 15.7
	0.5	Rapid	100.0 ± 0.0 ↓	100.0 ± 0.0 ↓	0.0 ± 0.0	0.0 ± 0.0
	1.0	Rapid	100.0 ± 0.0 ↓	100.0 ± 0.0 ↓	0.0 ± 0.0	0.0 ± 0.0
Thiomersal	0.0025%	Slow	44.0 ± 0.7 ↓	38.4 ± 1.3 ↓	56.9 ± 1.6	59.2 ± 1.6
	0.005%	Slow	43.1 ± 1.3 ↓	48.2 ± 2.1 ↓	40.8 ± 2.3	56.9 ± 4.8
	0.01%	Slow	65.0 ± 3.1 ↓	76.6 ± 3.6 ↓	24.6 ± 0.2	22.3 ± 3.4
	0.02%	Slow	100.0 ± 0.0 ↓	100.0 ± 0.0 ↓	0.0 ± 0.0	0.0 ± 0.0
Actinonin	0.5	No effect	5.0 ± 0.0 ↑	4.2 ± 0.1 ↑	107.4 ± 11.6	102.1 ± 15.0
	1.0	No effect	3.9 ± 0.6 ↓	3.6 ± 0.2 ↑	110.0 ± 8.7	101.2 ± 2.5
	2.0	No effect	6.6 ± 0.1 ↑	15.6 ± 0.1 ↑	99.3 ± 12.4	116.7 ± 21.6
	4.0	Rapid	20.4 ± 0.3 ↓	10.1 ± 0.2 ↓	102.7 ± 6.5	105.3 ± 3.8
	8.0	Rapid	56.4 ± 8.8 ↓	54.3 ± 4.8 ↓	103.0 ± 2.0	105.7 ± 0.3
DMSO (0.1%)		Rapid	11.9 ± 1.2 ↑	1.6 ± 0.1 ↑	109.2 ± 3.0	108.9 ± 20.0
DMSO (15.0%)		Rapid	62.7 ± 6.7 ↓	57.7 ± 11.2 ↓	110.0 ± 7.0	101.5 ± 5.1
Bacitracin	0.001	No effect	5.5 ± 0.4 ↑	6.2 ± 0.2 ↑	110.1 ± 4.7	ND
	0.01	No effect	1.4 ± 0.0 ↑	3.4 ± 0.0 ↑	110.4 ± 4.9	ND
	2.0	No effect	1.8 ± 0.1 ↑	4.6 ± 0.1 ↑	105.1 ± 9.5	ND
	145.0	No effect	1.5 ± 0.6 ↑	6.0 ± 1.0 ↑	105.4 ± 2.7	ND
	350.0	Slow	14.5 ± 1.2 ↓	3.8 ± 0.7 ↓	97.7 ± 3.9	ND
Puromycin	0.5	No effect	14.1 ± 0.8 ↑	4.0 ± 0.7 ↑	120.8 ± 2.8	ND
	1.0	No effect	7.2 ± 0.0 ↑	12.8 ± 0.6 ↑	109.1 ± 16.9	ND
	2.0	No effect	9.9 ± 0.8 ↑	8.8 ± 0.8 ↑	114.1 ± 14.3	ND
	5.0	No effect	6.0 ± 0.2 ↑	1.4 ± 0.1 ↑	107.1 ± 2.1	ND
	8.0	No effect	3.7 ± 0.2 ↑	6.5 ± 0.1 ↓	99.8 ± 9.2	ND

↑, CBF increase; ↓, CBF decrease; ND, not determined.

enkephalin aminopeptidase and dipeptidyl aminopeptidase with half-maximal inhibitory concentration (IC_{50}) values of 5.6, 0.4 and 1.1 μM, respectively. [19]. In this study, the effect of 0.5–8.0 mM on CBF was investigated. At a lower concentration range (0.5–2.0 mM), actinonin caused up to 15.0% CBF increase. In contrast, 4.0 and 8.0 mM of the compound resulted in approximately 20–50% cilioinhibition after 15–30 min exposure. A higher degree of ciliary inhibition occurred after 15 min exposure than after 30 min incubation due to the tendency of the cells to recover from the inhibition with time (without washing out the compound). Upon washout, the CBF of the cells fully recovered to pre-treatment values. Because actinonin was dissolved in DMSO, it was pertinent to rule out the possibility that the observed CBF reduction was not due to DMSO, as different concentrations of actinonin investigated contained 0.1–15.0% DMSO. Therefore, the effect of 0.1 and 15.0% DMSO on CBF was investigated. Whereas 0.1% DMSO caused a twofold CBF increase in comparison with 0.5 mM actinonin containing 0.1% DMSO, 15.0% DMSO alone resulted in 62.7 ± 6.7% CBF decrease compared with 56.4 ± 8.8% caused by 8.0 mM actinonin dissolved in 15.0% DMSO. The fact that 15.0% DMSO alone caused higher CBF reduction than 8 mM actinonin dissolved in 15.0% DMSO means that the observed CBF reduction was due to DMSO. Interestingly, both the cilioexcitatory and cilioinhibitory effect of DMSO (depending on the concentration investigated) was reversible. Previous studies have shown that up to 10% DMSO was suitable as a cryopreservative to preserve the ciliary activity of both human nasal and chicken trachea rings for a long period of time [20–21].

In contrast to the ciliotoxicity observed for bestatin and thiomersal, the CBF of cells exposed to 0.5–8.0 mM puromycin and bacitracin was not significantly reduced ($P > 0.05$) after acute exposure. However, significant, but reversible CBF reduction (≈15.0%) occurred only at a high concentration of bacitracin (145.0 mM). Therefore, because of lack of cilioinhibitory effect following acute exposures of relevant concentrations for nasal absorption enhancement of proteins and peptides, puromycin and bacitracin were used for subacute studies.

3.2. Mechanisms of ciliary inhibition

The molecular and cellular mechanisms by which the rate of dynein–microtubule interaction results in spontaneous ciliary beating is unclear. However, both phos-

phorylation and cytoplasmic calcium changes play a role in ciliary beating [22]. The pattern of dynein microtubule interactions suggests that CBF of the upper airway epithelium is modulated through two major pathways: beta 2-adrenoceptor pathway involving increased intracellular cyclic adenosinemonophosphate (cAMP) levels and muscarinic receptor mediated pathway involving production of prostaglandin, nitric oxide (NO) and cyclic guanosinemonophosphate (cGMP) [23]. Therefore, many substances have been reported to influence CBF by interfering with arachidonic acid metabolism, guanylate cyclase, protein kinase and intracellular ATP [24]. To elucidate the possible mechanisms through which bestatin, thiomersal and DMSO reduced the CBF, the influence of pre-incubating the cells with ATP (10^{-4} M), methylene blue (10^{-5} M), an inhibitor of guanylate cyclase, indomethacin (3×10^{-6}M), an inhibitor of cyclooxygenase enzyme that regulates arachidonic acid metabolism and H-7 (10^{-5} M), a protein kinase inhibitor on CBF were investigated [25].

It was found that the CBF of the cells (relative to control) was reduced by more than 35% after 15 min exposure to 0.07 mM bestatin alone, but this degree of CBF reduction was significantly reduced to 18–21 % after pretreatment with H-7, indomethacin, methylene blue and exogenous ATP (Fig. 1).

At higher concentrations of bestatin (0.1–1.0 mM), pretreatment with ATP, methylene blue, indomethacin and H-7 could not prevent instant ciliostasis seen at these concentrations. Similar results were obtained following a 30-min exposure. Based on the above data, one may postulate that bestatin reduced the CBF of human nasal epithelium by several mechanisms mediated by beta-adrenoceptors and/or muscarinic receptors, and possibly other unknown or non-specific mechanisms. The multiple mechanisms probably explain the instant ciliostasis seen with this compound.

For thiomersal and DMSO, CBF inhibition seems not to involve biochemical interference with arachidonic acid metabolism, guanylate cyclase, protein kinase and intracellular ATP utilization (Fig. 2A,B).

There was no statistically significant difference between the degree of ciliary inhibition caused by thiomersal and DMSO alone and that of pretreatment with ATP. In contrast, pretreatment with methylene blue, H-7 and indomethacin resulted in a higher degree of ciliary inhibition for both compounds. Bearing in mind that cAMP and Ca^{2+} regulate ciliary beat frequency by possibly regulating the rate at which the axoneme can use ATP, or the availability of ATP to the axoneme [26], studies with exogenous ATP provided indirect information on the involvement of cAMP and Ca^{2+}. Therefore, it can be suggested that the ciliotoxicity of thiomersal or cilioinhibition of DMSO does not involve inhibition of cAMP or intracellular calcium release, but may be due to presently unidentified mechanisms involving modulatory effects of other numerous dynein-associated accessory proteins other than calmodulin.

3.3. Subacute studies

Aspeden et al. [27] have shown that chronic effect of pharmaceutical excipients on CBF can be studied in vivo using guinea pigs. The present study demonstrated that subacute effect of drug excipients on CBF can also be screened using a cell culture model. Based on the observation that bacitracin and puromycin had no significant acute effect on the CBF at the concentrations used to enhance nasal drug absorption, these compounds were used for subacute studies. After exposure for 4 days, the CBF of the cells treated with both compounds was stable (≈95% of

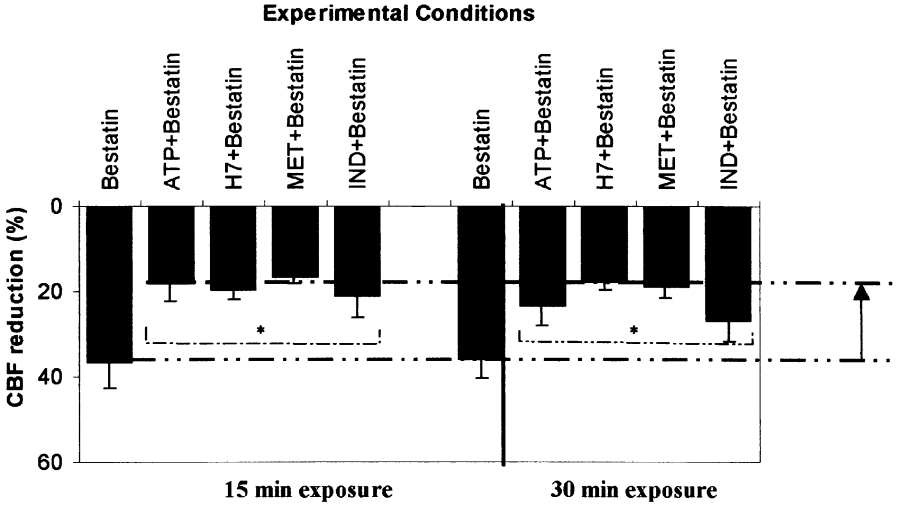

Fig. 1. Effect of preincubation with ATP, H-7, methylene blue and indomethacin on the cilioinhibition of bestatin. Data represents the mean ± SD, $n = 10$. Arrow direction depicts the reduction in the degree of CBF inhibition between bestatin alone (control) and in the presence of pharmacological agents after 15 min exposure. *Statistically significant reduction of cilioinhibition.

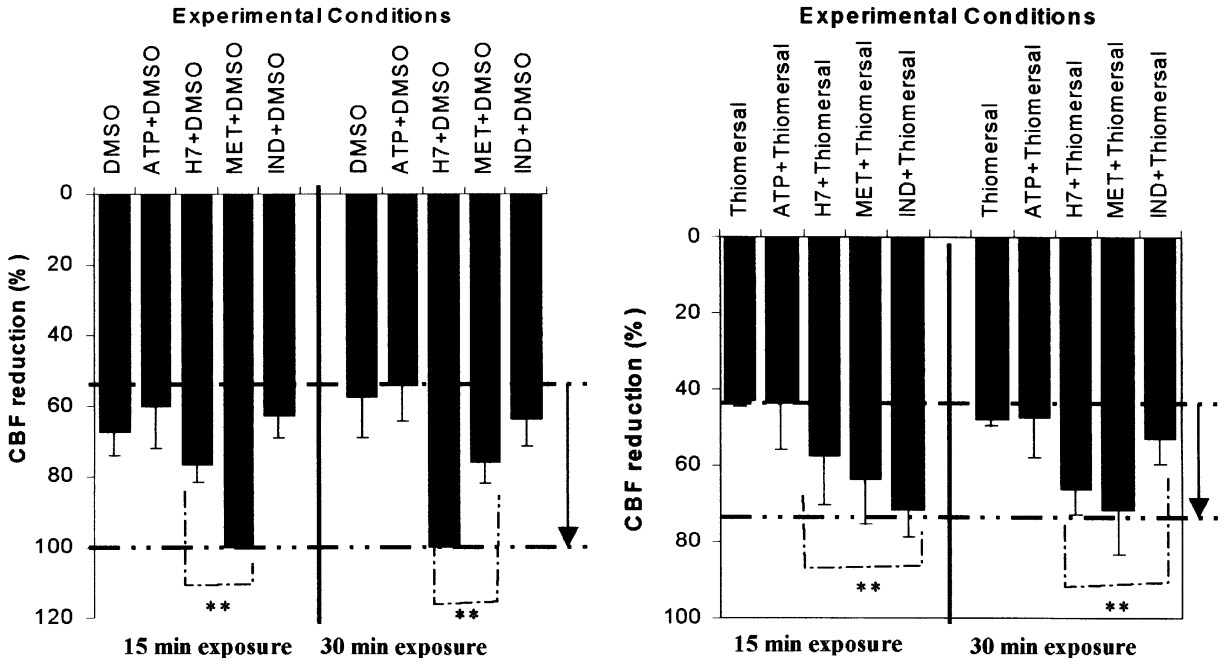

Fig. 2. Effect of pre-incubation with ATP, H-7, methylene blue and indomethacin on the cilioinhibition of thiomersal (A) and DMSO (B). Data represents the mean ± S.D., $n = 10$. Arrow direction depicts the increased degree of CBF inhibition between thiomersal and DMSO, respectively (control) and in the presence of pharmacological agents after 15 min exposure. **Statistically significant enhancement of cilioinhibition.

control) (Fig. 3). Further exposure for up to 7 days resulted in only a mild CBF reduction (<20.0%).

Bacitracin, used as a local antibiotic at a concentration of 0.5% (3.5 mM) has been shown to concentration-dependently enhance the nasal absorption of buserelin at a concentration range of 10–50 mM by inhibition of mucosal aminopeptidase activity [28]. Exposing the cells to 100 mM bacitracin for 7 days resulted in less than 20% CBF reduction. In a previous study, bacitracin (approx. 1000 mM) was reported to significantly impair the ciliary beating of chicken trachea [29]. Comparing the study with the results of the present study suggests that bacitracin is not

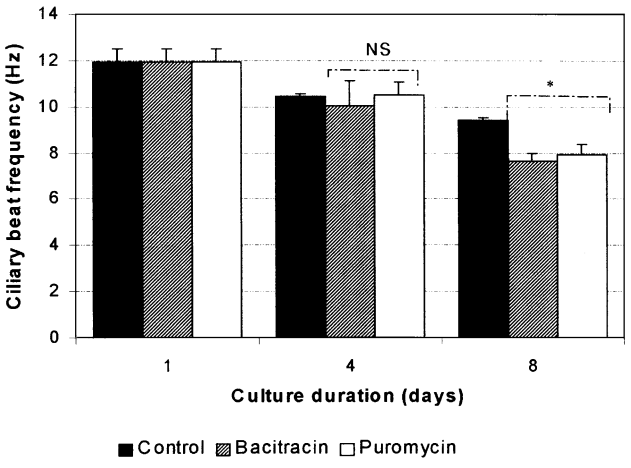

Fig. 3. Subacute effects of bacitracin (100 mM) and puromycin (1 mM) on CBF following 15 min daily exposure for 7 days. *Statistically significant cilioinhibition, NS, not significant.

cilioinhibitory when used within the concentration range suitable to enhance nasal absorption of proteins and peptides. Puromycin (1 mM) found to be effective as a nasal absorption enhancer [15,16] also had a mild effect on the CBF after subacute exposure. The fact that the concentrations of bacitracin and puromycin found to be effective as nasal absorption enhancer had only a mild effect on human CBF after subacute exposure and under in vitro conditions has provided more information on the safety of these compounds as nasal absorption enhancer from a ciliotoxicity perspective.

This study demonstrated that the sequential monolayer-suspension culture system is a suitable model to study both acute and subacute effects of pharmaceutical compounds on ciliary beat frequency and the mechanisms involved. Chronic studies are possible using this model provided large quantities of cells are cultured as loss of cells via repetitive washing is inevitable. Based on acute and subacute studies, it was evident that from a ciliary toxicity point of view, bacitracin, puromycin and actinonin are potentially more useful than bestatin and thiomersal for nasal absorption enhancement of proteins and peptides. This is buttressed by the fact that even at relatively high concentrations and under in vitro conditions (no mucus dilution and protection) bacitracin and puromycin caused only a mild cilioinhibition unlike bestatin and thiomersal that irreversibly stopped the ciliary beating at much lower concentrations after acute exposure. This study emphasized that the choice of protease inhibitors for nasal drug absorption enhancement should not only be based on efficacy and perturbation of nasal epithelial morphology

but also on ciliotoxicity given the fact that the mucociliary clearance is the most important nasal defensive mechanism.

4. Conclusions

This study demonstrated that the sequential monolayer-suspension culture system is a suitable model to study both acute and subacute effects of pharmaceutical compounds on ciliary beat frequency and the mechanisms involved. Chronic studies are possible using this model provided large quantities of cells are cultured as loss of cells via repetitive washing is inevitable. Based on acute and subacute studies, it was evident that from a ciliary toxicity point of view, bacitracin, puromycin and actinonin are more potentially useful than bestatin and thiomersal for nasal absorption enhancement of proteins and peptides. This is buttressed by the fact that even at relatively high concentrations and under in vitro conditions (no mucus dilution and protection) bacitracin and puromycin caused only a mild cilioinhibition unlike bestatin and thiomersal that irreversibly stopped the ciliary beating at much lower concentrations after acute exposure. This study emphasized that the choice of protease inhibitors for nasal drug absorption enhancement should not only be based on efficacy and perturbation of nasal epithelial morphology but also on ciliotoxicity given the fact that the mucociliary clearance is the most important nasal defensive apparatus.

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