Drug Development and Industrial Pharmacy, 2011; 37(12): 1455–1462

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2011.584537

informa
healthcare

RESEARCH ARTICLE

Safety assessment of thiolated polymers: effect on ciliary beat frequency in human nasal epithelial cells

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Abstract

Objective: The aim of this study was to investigate the nasal safety of gel formulations of thiolated polymers (thiomers) by assessing their effect on ciliary beat frequency (CBF) in human nasal epithelial cells.

Methods: Poly(acrylic acid) 450 kDa-cysteine (PAA-cys) and alginate-cysteine (alg-cys) were synthesized by covalent attachment of L-cysteine to the polymeric backbone. The cationic polymer chitosan-thiobutylamidine (chito-TBA) was synthesized by attaching iminothiolane to chitosan. CBF using was measured by a photometric system. CBF was measured before incubating the cells with test gels, during incubation and after washing out the polymeric test gels to evaluate reversibility of cilio-inhibition. The influence of viscosity on CBF was determined by using hydroxyethylcellulose (HEC)-gels of various concentrations.

Results: Ciliary beating was observed to be affected by viscosity, but cilia were still beating in the presence of a HEC-gel displaying an apparent viscosity of 25 Pa.s. In case of thiolated polymers and their unmodified control, a concentration-dependent decrease in CBF could be observed. PAA-cys, alg-cys, chito-TBA and their corresponding unmodified controls exhibited a moderate cilio-inhibitory effect, followed by a partial recovery of CBF when used at a concentration of 1%. Alg-cys 2% and chito-TBA 2% (m/v) gels exhibited severe cilio-inhibition, which was partially reversible. L-cysteine and reduced glutathione led to mild cilio-inhibition at concentrations of 3% (m/v).

Conclusions: Taking into account that dilution after application and cilio-modifying effects is usually more pronounced under *in vitro* conditions, thiomers can be considered as suitable excipients for nasal drug delivery systems.

Keywords: Ciliary beat frequency, thiolated polymer, alginate, poly(acrylic acid), chitosan, nasal toxicity

Introduction

Nasal drug administration has mainly been used to achieve a local effect within the nasal cavity; however, in the past decade, several systemic acting drugs have been marketed as well (e.g. for migraine treatment). Major advantages of the nasal route are a limited first-pass effect and a rapid onset of therapeutic effect. Nasal drug administration may also be an alternative to parenteral administration of drugs, such as the polypeptide hormone calcitonin, or to drugs which cannot be given orally. Nevertheless, there are still some drawbacks associated with nasal drug administration like a short residence time of drugs on the nasal mucosa due to ciliary clearance resulting in a limited time window for absorption. Polymeric excipients are known to be mucoadhesive; so, they are an option to prolong the residence time of drugs on the nasal mucosa[1]. Within the large family of polymers, thiolated polymers—designated thiomers—are a promising choice as they have been demonstrated to exhibit excellent mucoadhesive properties[2]. Due to the presence of thiol groups, they are able to form covalent bonds with cysteine-rich sub-domains of mucus glycoproteins. Additionally, thiomers were shown to exhibit a permeation enhancing effect. In addition, they exhibit enzymatic inhibitory properties reducing the pre-systemic metabolism of drugs on mucosal membranes[3,4].

The nasal mucosa, however, is not only a possible site for drug uptake but also an important barrier to avoid

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(Received 14 November 2010; revised 24 April 2011; accepted 26 April 2011)

absorption of toxic compounds from the environment[5]. Mucociliary clearance is one of the most important defense mechanisms of the respiratory tract[6]. Damage of the ciliary beating could result in poor mucociliary clearance, with subsequent upper and lower respiratory infection. The ciliary beat frequency (CBF) is often considered as an indicator for toxicity. Thus, it was the aim of this study to evaluate the impact of gel formulations based on thiolated polymers on the CBF to determine whether these excipients can be used as nasal drug delivery systems from the toxicological point of view. Within this study, the anionic polymers poly(acrylic acid) 450 kDa-cysteine (PAA-cys) and alginate-cysteine (alg-cys), and the cationic polymer chitosan-thiobutylamidine (chito-TBA) were chosen as representative thiomers for CBF-studies. The influence of viscosity was explored by using hydroxyethylcellulose (HEC) in increasing concentrations. Additionally, reduced glutathione (GSH), an additive in many formulations containing thiolated polymers, and L-cysteine were tested to examine whether these substances contribute to alterations in CBF of nasal cells.

Materials and methods

Materials

Poly(acrylic acid) with an average molecular weight of 450 kDa (PAA_{450}), sodium alginate with low viscosity (~250 cP), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC), L-cysteine free base, 2-iminothiolane.HCl (Traut's reagent), Ellman's reagent (DTNB, 5,5′-dithiobis(2-nitrobenzoic acid)), 2,4,6-trinitrobenzenesulfonic acid (TNBS), L-glutathione reduced form (GSH) and N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES) were obtained from Sigma-Aldrich, St. Louis, MO. Chitosan low viscosity was obtained from Fluka (Buchs, Switzerland) and HEC Natrosol© 250 M (HEC) from Hercules Inc. Aqualon, Wilmington, DE.

For cell culture preparation DMEM-Ham's F12 1:1 medium, penicillin/streptomycin solution and fetal calf serum (FCS) were purchased from Invitrogen Corp., Carlsbad, CA. Protease was obtained from Sigma, St. Louis, MO, Ultroser G serum from Ciphergen, Cergy, France and rat tail collagen type I at Upstate, Lake Placid, NY. Twelve-well cell culture plates and 25 cm^2 tissue flasks were purchased at Corning, Acton, MA. All other chemicals were of reagent grade and obtained from Sigma, St. Louis, MO, as well.

Polymer synthesis

The poly(acrylic acid) 450 kDa-cysteine and alginate-cysteine conjugates (PAA-cys and alg-cys) were synthesized according to a method described previously by our research group[7]. In brief, the covalent attachment of L-cysteine to alginate and neutralized PAA was achieved by the formation of amide bonds between the primary amino group of cysteine and the carboxylic acid group of the polymer. Therefore, the carboxylic acid moieties of the polymers were activated for conjugation by the addition of EDAC. After dialysis protected from light at 4°C to avoid oxidation of thiol moieties, the resulting polymer-cysteine conjugate solution were adjusted to pH 4.0 and lyophilized at −75°C condenser temperature at 4×10^{-4} mbar (Virtis, Gardiner, ME). Preceding freeze drying, the polymers were frozen at −70°C (Refco, Knoxville, TN). Both polymer conjugates were stored at 4°C until further use. Control polymers were prepared in the same way, however, without adding EDAC to the coupling reaction.

The synthesis of chitosan-4-thiobutylamidine conjugate (chito-TBA) has also been described previously[8]. In brief, a 1% (m/v) chitosan solution in 1% acetic acid (v/v) was stirred for 1 h. The pH was adjusted to 6.0 and 0.4% 2-iminothiolane HCl was added. After 12 h of incubation at room temperature under continuous stirring, the resulting conjugate was purified by extensive dialysis for five days. The resulting chito-TBA conjugate was dialyzed and lyophilized.

Degree of thiolation of the polymer conjugates

In order to test the influence of thiolated polymers on the CBF of nasal cells, thiol groups were linked to PAA and alginate by attaching L-cysteine to the polymers backbone due to the use of EDAC. In case of chitosan, Traut's reagent was the compound which was linked to the polymeric backbone.

The amount of free thiol groups immobilized on the polymer backbone, i.e. the degree of modification, was determined photometrically with Ellman's reagent quantifying free thiol groups. First, 0.5 mg of both the conjugates and control were hydrated in 500 µL of 0.5 M phosphate buffer pH 8.0. Then 500 µL Ellman's reagent (3 mg dissolved in 10 mL of 5.0 M phosphate buffer pH 8.0) was added. The samples were incubated for 2 h at room temperature protected from light. Thereafter, 300 µL of each sample was transferred into a microplate and the absorbance was measured at a wavelength of 450 nm using a microplate reader (Fluostar Galaxy, BMG, Offenburg, Germany[9]). The total amount of sulfhydryl groups fixed on the polymer, represented by the summation of free thiol groups and of oxidized thiol moieties available in form of disulphide bonds was quantified after reduction with $NaBH_4$[10]. The quantity of remaining unbound cysteine in the PAA_{450}-cys and alg-cys polymer gels was determined with TNBS. It reacts with the primary amino groups of cysteine in a nucleophilic aromatic substitution, developing an orange dye. The absorbance was measured at 450 nm using the Fluostar microplate reader[11].

Cell isolation and culture

Surgical specimens of human nasal epithelial tissue were obtained during elective surgery of nasal polyps. Human nasal epithelial cells were isolated according to the procedure described by Jorissen et al.[12], which includes pronase treatment and differential

attachment on plastic. The human nasal epithelial tissues were rinsed three times in saline solution (0.9% NaCl) and were enzymatically dissociated using 0.1% pronase solution in DMEM-Ham's F12 1:1 medium, supplemented with 50 IU/mL penicillin and 50 µg/mL streptomycin for a period of 16 h at 4°C. At the end of the pronase incubation, the large pieces of tissue were removed, and the protease activity was inhibited with 10% FCS. The cells were washed three times in DMEM-Ham's F12 1:1 medium, supplemented with 50 IU/mL penicillin, 50 µg/mL streptomycin and 2% Ultroser G by centrifugation (800 rpm, 5 min, 4°C). After the last centrifugation, the cell pellets were resuspended in 10 mL of the above mentioned complete medium and incubated for 1 h in a 25 cm^2 plastic tissue culture flask in a CO_2 incubator (5% CO_2–95% air, 37°C) to allow selective attachment of the contaminating fibroblasts and macrophages. The cell number was determined with a CASY cell counter (Schärfe System GmbH, Reutlingen, Germany). The cells were plated in rat tail collagen type I pre-coated 12-well plates (106 µl/cm^2) at a density of 5.3×10^4 cells/cm^2 in a final volume of 2 mL medium, and incubated at 37°C in atmosphere of 5% CO_2–95% air. The medium was changed 24 h after plating and subsequently every other day. Human nasal epithelial cell cultures were used for CBF measurements at day 6–7 after plating, when microscopically confluent layers, consisting of ciliated and non-ciliated cells were obtained[12,13].

Cell treatment and determination of CBF

All excipients were dissolved in basal cell culture medium pH 7.4, except for chitosan and chito-TBA which were dissolved in 1 M HEPES solution. All the solutions were freshly prepared on the day of the experiment. Chito-TBA and chitosan were dissolved in 1 M HEPES solution and pH was adjusted to 6.0 due to insolubility in cell culture medium.

Cells were preconditioned for 60 min at room temperature (22°C) and then CBF was measured to determine the initial value. After preconditioning, the cells were incubated with the different test solutions; between 20 and 25 min after exposure to the test compounds, CBF was measured. To investigate whether the effect on CBF is reversible after withdrawal of the polymeric excipient, CBF was also determined 60 min after rinsing the cell layer with cell culture medium. The CBF was determined according to a previously described method[14,15] with a computerized microscope photometry system consisting of a Laborvert FS light microscope and a Type MPV Combi photometer (Leitz, Jena, Germany) placed on a pneumatic vibration-absorbing table (Barry Controls, Ltd., Hersham, UK). CBF was measured at magnification 500; the diameter of the photosensitive field on the sample was 5 µm. The signal was photograph multiplied, digitalized at a sample frequency of 400 Hz, and transformed into a time-amplitude signal. A fast Fourier transformation analysis was performed using a period of 5.12 s. The highest peak of the first harmonic within this period was taken to represent the CBF. For each sample, CBF values of 10 different cells were detected. Every experiment was repeated at least 4 times. The measurements were always done with the same cell-fields. Results were presented as the mean values of these single measurements. The degree of CBF change caused by the tested substances was classified according to Merkus et al.[16] and Ugwoke et al.[5] as follows.

- no effect: less than 10% or statistically insignificant;
- mild: 10–20% cilio-stimulation: inhibition and statistically significant;
- moderate: 20–50% cilio-stimulation: inhibition and statistically significant;
- severe: greater than 50% and statistically significant.

Reversibility of effect after washing out the test substance was determined with the following equation[5]:

$$\%\,\text{Reversibility} = \frac{\text{CBF after washing} - \text{CBF following treatment with test substance}}{\text{Control CBF} - \text{CBF following treatment with test substance}} \times 100 \quad (1)$$

Reversibility was classified as follows:

% Reversibility greater than 75: reversible
% Reversibility 25–75: partially reversible
% Reversibility less than 25: irreversible

Rheological studies

These studies were performed on a cone-plate viscosimeter type Physica Rheolab MC1 from Paar Physica, Graz, Austria. HEC was hydrated in cell culture medium in concentrations of 0.25, 0.5, 1, 2, 3 and 4% (m/v) and allowed to equilibrate at 22°C on the plate for 3 min before measurement. Every apparent viscosity was determined at a controlled shear rate of $D = 10\ s^{-1}$.

Statistical data analysis

Statistical data analysis was performed using ANOVAs with $p < 0.05$ as the minimal level of significance. All values will be expressed as the means ± standard deviation.

Results

Characterization of the different thiomers

The lyophilized PAA-cys and alginate-cys conjugates contained between 3 and 6 µmol unbound L-cysteine (data not shown). The amount of unbound iminothiolane was not determined as it was purified by extensive dialysis. The amount of thiol groups covalently attached to the polymers is reported in Table 1.

Determination of the influence of viscosity on CBF

For the evaluation of the viscosity on CBF, HEC was chosen, because it is non-ionic and displays almost

pseudoplastic behavior. Furthermore, it is declared to be harmless from a toxicological point of view, stable and inactive (Tien et al., 2005). Thus, change in CBF should only result from different viscosity of HEC gels used at varying concentrations. Due to increasing concentrations, the increase in apparent viscosity can be described by following equation: $y = 2.1714x^2 - 2.4879x + 0.645$. For a 2% (m/v) HEC-solution in cell culture medium a viscosity of 4043 ± 366 mPa·s was determined, which is in good correlation with the specifications of the manufacturer who specifies the viscosity of a 2% (m/v) HEC-solution in water (20°C, $D = 10\ s^{-1}$) around 5000 mPa·s (Figure 1A).

The decrease in CBF as a result of increasing HEC-gel concentrations applied on the cells was approximately linear. Gels consisting of 3 and 4% (m/v) HEC in cell culture medium could not be removed from the monolayer; therefore, recovery of CBF could not be determined for these concentrations. As shown in Figure 1B, ciliary beating was not completely inhibited when incubated with a gel characterized by a viscosity of 25,000 mPa·s. Furthermore, it was demonstrated that HEC of moderate viscosity does not have a sustained cilio-inhibiting effect, as CBF recovered almost completely after having washed out the test solutions.

Influence of thiolated polymers on CBF

The effect of PAA-cys was dependent on the concentration to which the cells were exposed. A 0.5% (m/v) solution caused no statistically significant effect on ciliary beating, whereas a mild and moderate cilio-inhibiting effect was obtained at a concentration of 1 and 2% (m/v), respectively. Rinsing the cells after a 30-min exposure period of incubation led to CBF which can be described as reversible for the 1% (m/v) solution and partially reversible for the 2% (m/v) solution. These results are illustrated in Figure 2. In case of alginate-cys, there was no significant change in CBF observed when cells were incubated with a 0.5% (m/v) solution. Moderate cilio-inhibition was observed at a concentration of 1% (m/v) alginate-cys, whereas the presence of unmodified alginate appeared to result in mild cilio-inhibition (Figure 3).

Two percent of (m/v) alginate-cys gels showed an even more pronounced effect, at one sample ciliostasis was recorded. Though there was severe cilio-inhibition, reversible recovery of ciliary beating could be observed. It can be seen that alginate-cys causes 50% degree in CBF by half the concentration of alginate, but after washing out the test solutions, there was no significant difference between the thiolated polymer and its unmodified control.

Chitosan-TBA and unmodified chitosan, representing cationic polymers, showed almost the same effect on the cells as the anionic polymers alginate and alginate-cys. Chito-TBA gel 0.5% (m/v) had no significant effect on ciliary activity. The 1 and 2% gel of chito-TBA displayed a moderate and severe cilio-inhibitory effect, respectively, and both concentrations showed a partially reversible effect (Figure 4). The CBF was partially reversible for all chitosan solutions.

To verify whether the influence of the various thiolated polymers on CBF can be attributed to L-cysteine, it was investigated in a separate experiment. Results are depicted in Figure 5 demonstrating that L-cysteine (3%, m/v) lowered CBF to 87% of the control value and that the effect

Table 1. Amount of thiol groups, disulphide groups and the total amount of thiol groups (µmol/g polymer) attached to each polymeric backbone.

Thiomer	-SH (µmol/g)	-S-S-(µmol/g)	∑-SH (µmol/g)
PAA_{450}-cys	549.5 ± 4.2	89.6	639.1 ± 18.0
Alg-cys	381.4 ± 43	47.2	428.6 ± 14.7
Chito-TBA	329.1 ± 26	44.9	374.0 ± 15.0

Free thiol groups were determined by the Ellman's test. Determination of the total amount of thiol groups was performed with the Ellman's test after pretreated with sodium borohydride to cleave disulphide bonds ($n = 3$, ± SD).

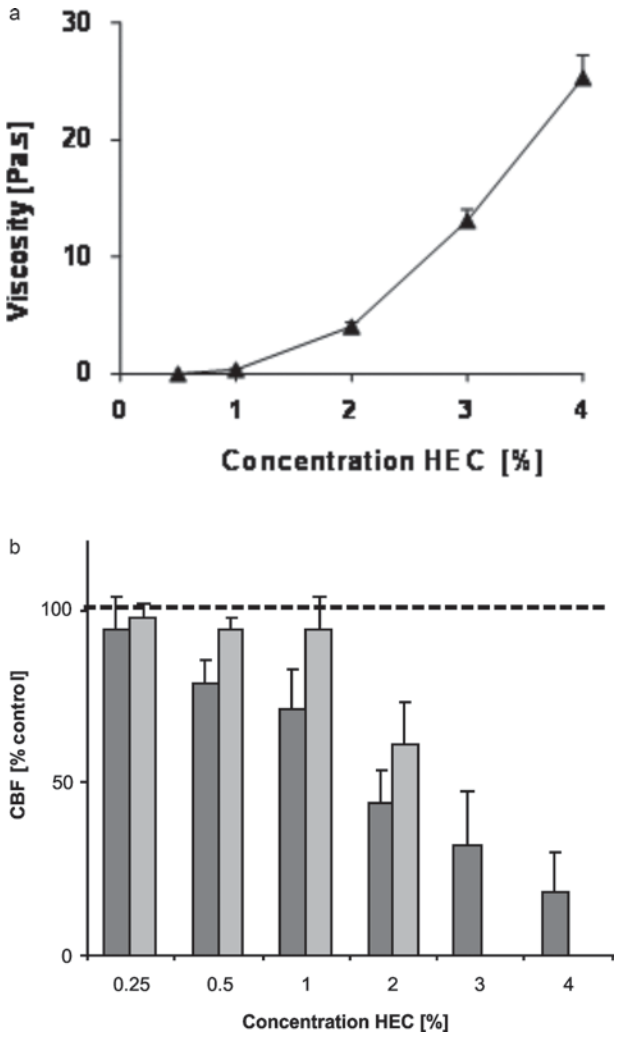

Figure 1. (A) Apparent viscosity of different hydroxyethylcellulose (HEC) gels. Studies were performed at a controlled shear rate of $D = 10\ s^{-1}$ at 22°C ($n = 3$, ± SD). (B) Effect of different concentrations of hydroxyethylcellulose gels (HEC) on CBF in comparison to control. Dark grey bars indicate measurement after 20 min of exposure and light grey bars indicate CBF 60 min after washing out the HEC gels ($n = 3$, ± SD).

was fully reversible. At lower concentrations of L-cysteine, no significant effect could be recorded (data not shown). Figure 5 also shows the influence of GSH, an excipient which is widely used in combination with thiolated polymers for permeation enhancing drug carrier systems. The preliminary used concentration of 0.5% (m/v) had no effect on CBF (data not shown) and a 3% (m/v) solution was determined to have a mild cilio-inhibitory effect[17].

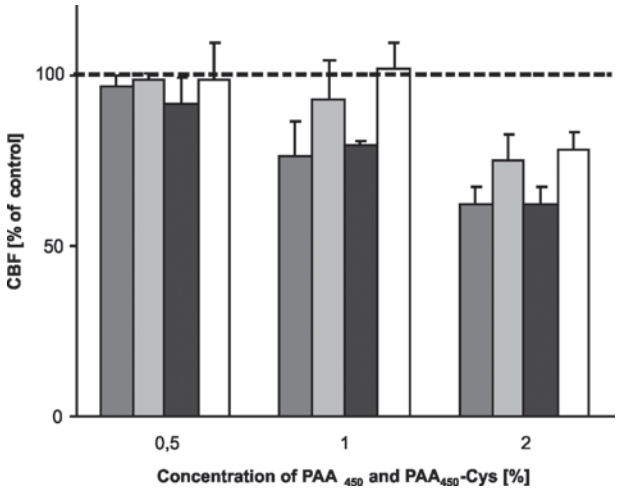

Figure 2. Effect of different concentrations of PAA-cys gels and its unmodified control on CBF. Dark grey bars indicate measurement of unmodified PAA after 20 min of exposure, light grey bars indicate CBF of unmodified PAA 60 min after having washed out the test solutions, black bars indicate measurement of thiolated PAA-cys after 20 min of exposure and white bars indicate CBF of thiolated PAA-cys 60 min after washing out the test solutions ($n = 3$, ± SD).

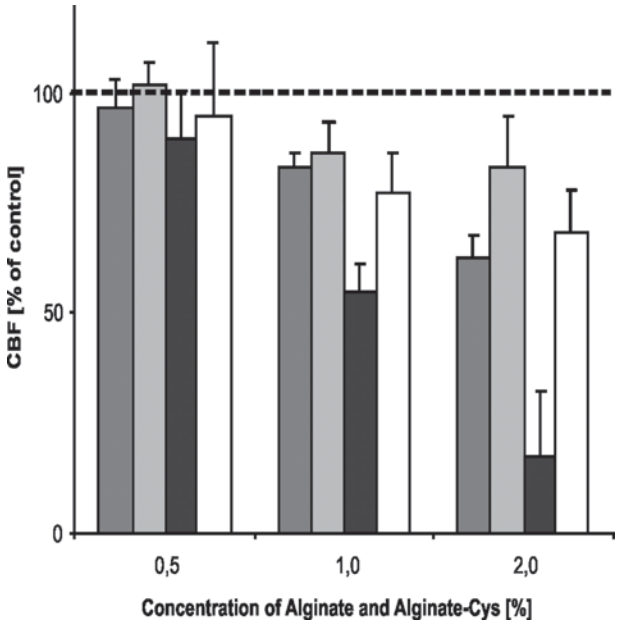

Figure 3. Effect of different concentrations of alginate-cys solutions and its unmodified control on CBF. Dark grey bars indicate measurement of unmodified alginate after 20 min of exposure, light grey bars indicate CBF of unmodified alginate 60 min after having washed out the test solutions, black bars indicate measurement of thiolated alginate-cys after 20 min of exposure and white bars indicate CBF of thiolated alginate-cys 60 min after washing out the test solutions ($n = 3$, ± SD).

Discussion

Polymers play an important role in nasal drug delivery. Polymers such as hydroxypropyl cellulose, carboxymethylcellulose, microcrystalline cellulose, hypromellose or hyaluronic acid have been used as excipients in

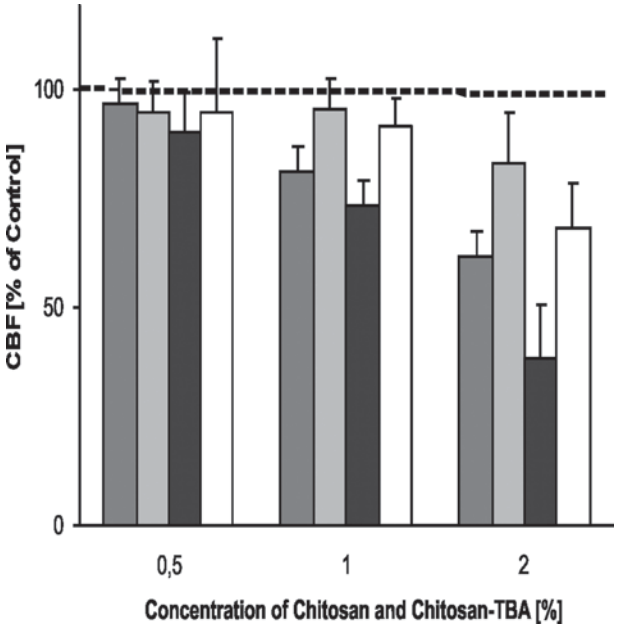

Figure 4. Effect of different concentrations of chitosan-TBA solutions and its unmodified control on CBF. Dark grey bars indicate measurement of unmodified chitosan after 20 min of exposure, light grey bars indicate CBF of unmodified chitosan 60 min after having washed out the test solutions, black bars indicate measurement of thiolated chitosan-TBA after 20 min of exposure and white bars indicate CBF of thiolated chitosan-TBA 60 min after washing out the test solutions ($n = 3$, ± SD).

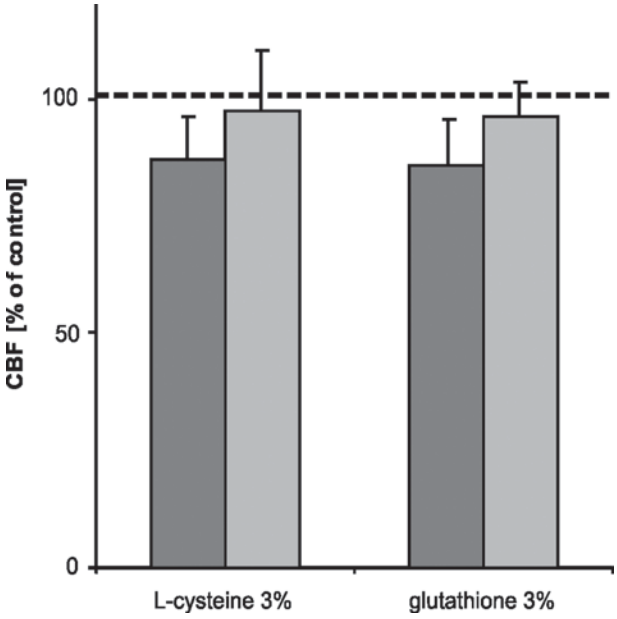

Figure 5. Effect of 3% (m/v) L-cysteine and 3% (m/v) glutathione solutions on CBF in comparison to control. Dark grey bars indicate measurement of L-cysteine and glutathione after 20 min of exposure, light grey bars indicate CBF 60 min after washing out the test solutions ($n = 3$, ± SD).

pharmaceutical formulations[18,19]. Due to bioadhesive properties, the polymers adhere to the nasal mucosa. The formation of nasal gel avoids the foreign body sensation. In addition, they act as release controlling matrix, thus allowing sustained drug delivery. However, only few polymers have been tested toward their influence on CBF so far.

CBF and mucociliary clearance (MCC) go hand in hand and a drug or excipient which is able to modify CBF has, therefore, also an impact on MCC. If MCC is decreased, infections of lower airway regions might occur. Additionally, the residence time of a drug might be prolonged due to decreased MCC. Drugs can also directly act on CBF, which is dependent on Ca^{2+}, cAMP and ATP levels[20].

For this study, a well-established primary human nasal cell culture system was used[21,22]. Agu et al.[21] confirmed the reliability of the *in vitro* cell culture system used for CBF determination. Nasal cell culture models are generally accepted for screening the influence of drugs and excipients on CBF[23]. The local toxicity of the different excipients measured by CBF *in vitro* is probably too sensitive[24]. *In vitro*, the excised ciliated tissue is totally immersed in the test formulation, whereas *in vivo* the viable ciliated epithelial cells are protected by the physiological mucus barrier. Thus, the effect of possibly noxious substances can be more pronounced *in vitro* than *in vivo*.

Nevertheless, *in vitro* CBF-studies became a routine procedure when developing nasal drug delivery systems. The nasal cell culture model used for this study exhibited stable ciliary beating around 8–11 Hz at room temperature which correlates with other studies[25,26]. Furthermore, in nasal monolayer cultures an increase in cell numbers within the 1st week, resulting in confluent monolayers was investigated. On collagen gels, the epithelial cells grew to confluency and become squamous[12]. After 6 days, all cells were attached to the collagen gel and a monolayer of ciliated and non-ciliated cells was obtained. Kissel et al.[27] showed growth of human nasal epithelial cells in culture to a confluent monolayer within 6–10 days. These cultures expressed microvilli and actively beating cilia.

Besides the factors mentioned so far, CBF is also dependent on external parameters like viscosity, temperature, osmotic pressure and pH. Within this study, temperature was maintained at room temperature (22°), whereas osmolality and pH were kept constant through the use of cell culture medium and HEPES solution, respectively. The HEPES solution by itself did not influence ciliary activity as it was shown in a previous study[28].

Thiomers can provide a concentration-dependent cytotoxicity. Therefore, the influence of viscosity was determined by using HEC gels because of its nontoxic label, its stability and inactivity[29]. It has been revealed that there is an inverse relationship between CBF and viscosity. However, upon increasing the concentration, CBF decreases linearly, whereas the increase in viscosity is non-linear. It could be demonstrated that CBF could be significantly reversible reduced due to highly viscous polymers.

The knowledge gained by these studies was used to explain the decrease of CBF due to anionic and cationic thiolated polymers. Two percent (m/v) gels of PAA-cys and unmodified PAA had an apparent viscosity of 100 mPa·s at pH 7.4 in cell culture medium (data not shown) and CBF decreased to 62% of the initial value under the influence of this gel. Furthermore, 2% (m/v) gels of HEC showed a viscosity of 4500 mPa·s and CBF decreased to only 45% of the initial value. Thus, probably not only gel-viscosity can be responsible for the cilio-inhibitory effect of PAA-cys. L-cysteine was found not to be responsible for this effect. It was noticed that PAA-cys is the thiolated polymer exhibiting the highest concentrations of thiol groups and also having the lowest cilio-inhibition. Furthermore, by comparison with thiolated polymers, cysteine and glutathione showed a more than 10 times higher amount of thiol groups and a higher cilio-friendly effect. Ugwoke et al.[5] performed CBF-studies with Carbopol 971P, which is poly(acrylic acid) cross-linked by allyl penta erythritol, on a human nasal cell culture model as well. They detected a reversible decrease of 50% in CBF at a concentration of 0.5% (m/v). Although alg-cys and its unmodified analogue differed in their impact on CBF, CBF recovered to almost the same level after washing out the excipients. Since a 2% (m/v) gel of alginate had an apparent viscosity of 1.5×10^{-4} mPa·s rheological effects cannot be the only reason for a decrease in CBF. Probably, alginate and alg-cys are capable of complexing Ca^{2+} ions and depletion in Ca^{2+} levels may result in cilio-inhibition. Chito-TBA and unmodified chitosan are the only cationic polymers used in this study. Haffejee et al.[30] investigated the influence of chitosan and they detected a reduction in CBF of 12.7% for chitosan HCl (1%, w/v). These results correspond with the results obtained in our study. Obviously, modified alg-cys and chito-TBA exhibited a higher decrease in CBF in comparison to the unmodified polymers which can be ascribed to an increased cytotoxicity.

In a previous study[31], the toxicity profile of chitosan-TBA was evaluated on L-929 mouse fibroblast cells utilizing two different bioassays. In test compound concentrations as low as 0.025%, a negligible cytotoxicity was observed, whereas at a concentration of 0.25%, an increase in cytotoxicity occurred. According to the results, a strong concentration-dependent cytotoxic effect could be identified. Comparing all (thiolated) polymer gels used in the CBF-studies, PAA-cys exhibited the lowest ciliary toxicity (Figure 2). Furthermore, in a previous study, PAA and PAA-glutathion showed no significant cell toxicity[32].

Thiomer gels and glutathione—when used as permeation enhancers—are mostly used in concentrations of 0.5%. Furthermore, at this concentration a stabilizing effect, for example of antisense oligonucleotides, was

observed[33]. As it was shown at this concentration, the thiomers displayed no cilio-inhibition.

The safety of chitosan-TBA was also illustrated by Krauland et al.[34]. Nasal microparticulate delivery systems for insulin based on chitosan-TBA were generated via a new precipitation-micronization technique and tested *in vivo*. After nasal administration to conscious, non-diabetic rats, chitosan-TBA microparticles comprising insulin led to a more than 1.5-fold higher bioavailability and a more than 7-fold higher pharmacological efficacy than insulin-loaded unmodified chitosan microparticles. Therefore, thiolated polymers such as chitosan-TBA or PAA-cys in a concentration range of 0.5–1% seem to have substantial higher potential for nasal administration than the unmodified ones.

Conclusion

Thiolated polymers are promising excipients for nasal drug delivery systems and therefore, this study examined the influence of these thiomers on CBF. *In vitro* CBF-studies are commonly used to evaluate the toxicological profile of nasally administered drugs. As test system, human nasal cell cultures were used, and CBF was recorded photometrically.

Thiolated polymers exhibited no significant change in CBF when used at 0.5% (m/v) gels, a concentration at which many *in vitro* tests are performed. At higher concentrations, mainly moderate cilio-inhibition could be observed, which was partly reversible. From this point of view—keeping in mind that cilio-inhibtion is less pronounced in the *in vivo* situation due to local dilution—thiolated polymers can be recommended as excipients for nasal drug delivery.

Declaration of interest

The Austrian Nano-Initiative co-financed this work as part of the Nano-Health project (no. 0200), the subproject NANO-N-0204 being financed by the Austrian FWF (Fonds zur Förderung der Wissenschaftlichen Forschung) (Project no. N-0204-NAN).

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