European Journal of Pharmaceutics and Biopharmaceutics 80 (2012) 156–163

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Development of a novel model for comparative evaluation of intranasal pharmacokinetics and effects of anti-allergic nasal sprays

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ARTICLE INFO

Article history:
Received 26 May 2011
Accepted in revised form 7 September 2011
Available online 14 September 2011

Keywords:
Fluticasone propionate
Budesonide
Azelastine
Tissue retention
Nasal spray
IL-8

ABSTRACT

For locally acting drugs, an extended residence time in the nasal cavity is desirable and related to a prolonged effect. We sought to develop a model for comparative determination of intranasal pharmacokinetics. We embedded human respiratory tissue into a solid matrix and coated the surface with artificial nasal fluid. Nasal spray suspensions of fluticasone propionate (FP) and budesonide (Bud) as well as a solution of azelastine hydrochloride (AZ) were applied onto the surface and removed after 30 min to simulate mucociliary clearance. As exemplary anti-inflammatory measure, we evaluated the inhibition of IL-8 release from epithelial cells. FP and Bud were initially bound to the same extent to the tissue gel while AZ displayed a more 4-fold higher binding than FP or Bud. After equilibrium with plasma, approximately 5-fold higher tissue concentrations of AZ compared to FP and 77-fold higher levels in relation to Bud were determined. This tissue retention revealed an excellent correlation with the volume of distribution of the respective drugs ($r = 0.9999$, $p \leqslant 0.05$). The inhibitory effect of FP on IL-8 release was approximately 5-fold more pronounced compared to AZ. The present model realistically mirrors conditions *in vivo* where solubility and tissue absorption of intranasally applied drugs compete with mucociliary clearance mechanisms.

1. Introduction

Intranasal glucocorticoids play a prominent role in the treatment of allergic rhinitis as they potently reduce nasal inflammation and nasal hyperreactivity [1]. Besides, topical glucocorticoids oral or local antihistamines are widely used as inhibitors of the allergic response. For locally acting anti-allergic drugs, an extended residence time in the nasal tissue is related to a prolonged pharmacologic activity. An additional advantage is the slow distribution into systemic circulation resulting in low plasma concentrations [2] and therefore a low risk of systemic adverse effects. The pharmacokinetic behavior such as dissolution in nasal fluid, diffusion, and retention in the nasal tissue of those drugs governs their presence at the therapeutic target site.

Though *in vivo* studies have the greatest human relevance and allow both the determination of drug pharmacokinetics and pharmacodynamic effects, usually high doses and sensitive analytical methods are required to describe the glucocorticoid concentrations and time courses in plasma. Radionuclide imaging studies in humans allow conclusions about drug deposition and retention in the nasal cavity [3]. However, this type of studies is too elaborate for routine use in drug development and for comparative analysis of drug pharmacokinetics.

Besides clinical evaluations, various *in vitro* test systems have been employed to study drug pharmacokinetics after intranasal application. For assessment of nasal spray deposition pattern, nasal cavity replica are used [4,5]. To elucidate transport and metabolism of drugs in the human, nasal epithelium cell culture settings are frequently employed [6–8]. These cell culture models allow a rapid and comparative screening of drug absorption and a thorough evaluation of transport or absorption mechanisms. Additionally, the ciliary beat frequency can be analyzed simultaneously to the transepithelial transport to determine potential cilio-toxic effects [9]. Recently, the cell culture approach was advanced to a three-dimensional reconstructed nasal mucosa using a collagen matrix and co-culture of two cell types [10]. A major limitation of a cell culture setting is that it is usually performed under static conditions although growth of human epithelial cells in a perfusion system has been described as well [11]. Another model that allows the determination of transport and metabolism processes is excised nasal mucosa tissue [12,13]. The bovine tissue was viable up for several hours and was suggested to offer a high potential for nasal permeation and metabolism studies in drug development.

We previously compared the tissue binding and retention of various glucocorticoids in human lung [14–16] and nasal tissue

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doi:10.1016/j.ejpb.2011.09.003

[17,18]. Therefore, we used a simple test system employing cut human tissue pieces that were incubated with glucocorticoid-containing buffer solutions until the tissue was drug-saturated. After transfer of the pieces into human plasma, the drug fraction retained in the tissue after one hour of equilibrium was determined. Though this test system was very simple, the tissue-retained drug concentrations correlated well with *in vivo* data determined in lung tissue after inhalation [19] or in nasal tissue after intranasal application [20].

More recently, we determined the dissolution behavior of glucocorticoids in artificial nasal fluid and found vast differences between the individual compounds as well a strong influence of the proteins in the nasal fluid on the solubility of most lipophilic glucocorticoids fluticasone propionate, mometasone furoate, and fluticasone furoate [18]. However, the simple test system we used did not allow us to elucidate the processes of the compounds' dissolution and tissue binding in a single experimental run.

The purpose of the present study was to develop a suitable model for comparative determination of intranasal pharmacokinetics of drugs applied locally to the nasal mucosa. This model was supposed to allow the use of commercially available drug formulations, to account for the dissolution of drug particles in nasal fluid in case of nasal spray suspensions and to simulate mucociliary clearance processes. Since the mucociliary transport removes drug particles from the respiratory region in the nasal cavity [3,21–23], some drugs might not have a sufficient chance to dissolve and diffuse into the mucosa. In this context, our aim was to determine whether a glucocorticoid with a rather high aqueous solubility but low tissue binding affinity would outperform a corticosteroid with very low water solubility and high tissue binding.

For the first proof-of-concept experiments, we chose budesonide that is fairly well water-soluble and of which high concentrations are already dissolved in the aqueous supernatant of a commercial nasal spray [18] and the poorly soluble fluticasone propionate. Since we already had tissue binding and retention data on these compounds, they were the most suitable model drugs. As model system, we prepared a "tissue stripe" that contained respiratory tissue pieces embedded into a solid matrix to allow handling and e.g. extensive washing of the tissue gel. Though we previously observed some differences in tissue binding and retention of drugs between human lung and nasal tissue [14], we used lung instead nasal tissue in the present experiments since the availability of human nasal tissue pieces is very low. In case of reasonable and consistent results with our new model, we intended to test a drug from another class in our system, more specifically, a topically used H_1 receptor antagonist.

Furthermore, we compared the effects of the tissue-bound fractions of the respective drugs to demonstrate that our model also allows pharmacodynamic investigations. Therefore, we exemplarily determined the anti-inflammatory activity of two of the compounds upon inhibition of IL-8 secretion from human epithelial cells after an inflammatory stimulus. The chemokine IL-8 has been found in nasal secretions of patients with allergic rhinitis after allergen challenge [24], and the IL-8 concentration revealed a significant correlation with the symptom score [25].

2. Materials and methods

2.1. Chemicals and Reagents

Azelastine–HCl (AZ), Budesonide (Bud), and dimetindene maleate were purchased from Sigma–Aldrich–Chemie (Taufkirchen, Germany). Amcinonide was obtained from Cyanamid (Wolfratshausen, Germany). Fluticasone propionate (FP) was a generous gift from GlaxoSmithKline (Greenford, England). Nasal glucocorticoid sprays (FP, Flutide® Nasal [GlaxoSmithKline]; Bud, Budes® [Hexal]) and antihistamine nasal spray (AZ, Vividrin® akut [Bausch + Lomb]) were obtained from a local pharmacy. Mucin (from porcine stomach, type II) and bicinchonic acid were purchased from Sigma–Aldrich–Chemie (Taufkirchen, Germany), bovine serum albumine (BSA), and N-(2-hydroxyethyl)piperazine-N′-2-ethanesulfonic acid (HEPES) from Gerbu (Heidelberg, Germany). Diethylether (p.a. quality) was obtained from Fluka (Buchs, Switzerland) and acetonitrile (HPLC gradient grade) from VWR (Ismaning, Germany). Rotiphorese 30 Gel (30% Acrylamide-, Bisacrylamide-solution 37.5:1), Tris (Pufferan®, ⩾99.9%, p.a. Tris-(hydroxymethyl)-aminomethane, Tris), Tris–hydrochloride (Pufferan®, ⩾99%, p.a. Tris-(hydroxymethyl)-aminomethanehydrochloride, Tris–HCl), ROTI® Stock 20% SDS (20% solution sodium dodecylsulfate, SDS), Ammonium peroxodisulfate (⩾98%, p.a., APS), and TEMED (N,N,N′,N′-Tetramethylethylenediamine,1,2-Bis(dimethylamino)-ethane; 99%, p.a.) were purchased from Roth (Karlsruhe, Germany). Phosphate buffered saline (PBS) salts were obtained from Biochrom AG (Berlin, Germany). Water from a Millipore® water purification unit was used. All other chemicals were obtained from Merck KGaA (Darmstadt, Germany).

2.2. Buffer solutions

PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4) was adjusted to pH 7.4. Krebs–Ringer–HEPES buffer (pH 7.4) consisted of 118 mM NaCl, 4.84 mM KCl, 1.2 mM KH_2PO_4, 1.18 mM $MgSO_4 \times 7H_2O$, 2.44 mM $CaCl_2 \times 2H_2O$, and 10 mM HEPES. Resolving gel buffer consisted of Tris–base 0.85 M and Tris–HCl 0.15 M.

2.3. Cell culture reagents

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, L-glutamine, nonessential amino acids (NEA), Trypsin/EDTA (0.05/0.02% in calcium- and magnesium-free phosphate buffered saline), and Trypan blue (0.5% in physiological saline) were purchased from Biochrom AG (Berlin, Germany). Lipopolysaccharide (LPS, from Salmonella minnesota RE 595, Sigma–Aldrich–Chemie, Taufkirchen, Germany) was dissolved in cell culture medium.

2.4. Cell culture conditions of lung epithelial cells

The human lung adenocarcinoma epithelial cell line A549 was purchased from DSMZ (Braunschweig, Germany). Cells were maintained in DMEM containing 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM glutamine, 1 mM NEA and were grown as monolayers in 75 cm^2 tissue culture flasks (Nunc, Roskilde, Denmark) at 37 °C in a humidified atmosphere of 5% CO_2. Medium was changed every second day. At confluence, the cells were removed from the flask by trypsin/EDTA treatment. For experiments, cells were seeded in six-well plates at a concentration of 2×10^5 cells/well and grown to subconfluence.

2.5. Preparation of artificial nasal fluid (ANF)

The preparation of artificial nasal fluid (ANF) was performed according to the procedure described earlier [18]. Mucin was dispersed in PBS and shaken in an ultrasonic bath for a total of 2 h. Thereafter, the homogenous mucin dispersion was centrifuged for 10 min at 3345*g* at 15 °C (Megafuge 1.0R, Biofuge A, Kendro-Heraeus, Berlin, Germany). The supernatant obtained was centrifuged again for 15 min at 18,000*g* at 15 °C (Microfuge® 22R Centrifuge, Beckman Coulter, California, USA). The protein concentration of the supernatant was determined according to the

method of Smith et al. [26]. In the following, the protein concentration of the mucin dispersion was adjusted with BSA to 8 mg/mL according to total protein levels of human nasal mucus [27]. ANF was adjusted to pH 6.5 with 1 N NaOH, and PBS was adjusted to pH 6.5 with 1 N HCl. ANF and PBS were stored in aliquots at −80 °C.

2.6. Source and handling of human specimen

Human lung tissue specimen was obtained from patients from the Thoraxzentrum Unterfranken with bronchial carcinomas scheduled for lobectomy who gave informed consent. The use of resected human lung tissue was approved by the Ethics Committees of the Medizinische Fakultät of the Eberhard-Karls-Universität Tübingen and the Universität Würzburg. Only cancer-free tissue was used for the experiments. Tissue samples from 5 patients were pooled for the experiments. None of the patients was treated with glucocorticoids for the last two weeks prior to surgery. Immediately after resection, the tissue was frozen and stored at −80 °C until usage. Tissue was washed in Krebs–Ringer–HEPES buffer (pH 7.4) and sliced into pieces of approximately 1 mm^3. Human plasma and erythrocyte concentrate were obtained from the Department of Transfusion Medicine and Immune Haematology, Würzburg, Germany. Plasma samples from at least three patients were pooled, shock frozen in liquid nitrogen, and stored at −80 °C until usage. Erythrocyte concentrate was stored at 6 °C.

To determine packed cell volume, the erythrocyte concentrate was centrifuged for 10 min at 10,000*g* at 6 °C. The volume of the supernatant was specified to adjust whole blood with a hematocrit of 40%.

2.7. Preparation of polyacrylamide-tissue gel

The polyacrylamide gel consisted of acrylamide 30% 0.667 mL, resolving gel buffer 0.750 mL, Millipore®-water 0.546 mL, 10% SDS solution 50 µL, 10% APS solution 50 µL, and TEMED 4 µL. A petri dish was used to form the gel with a diameter of 3.75 cm. Before adding TEMED to the gel preparation, sliced tissue pieces were spread out at the glass surface. To ensure a homogenous distribution, tissue pieces were mixed within the gel matrix thoroughly. The gel matrix was covered with a layer of Millipore® water and allowed for polymerization. For comparison, a gel without embedded tissue pieces was casted for every experiment.

2.8. Adsorption of drugs to polyacrylamide-tissue gel

The polymerized gel was transferred to a round ground-joint glass dish with ground-glass lid. Only glass laboratory ware was used to avoid any nonspecific binding of the highly lipophilic compounds to plastic material. A thin layer of PBS buffer at the bottom of the glass dish maintained the gel humidity. To determine drug adsorption, a defined dose (45 µg) of the commercially available nasal formulation was dispersed in ANF. Immediately, 500 µL containing 9 µL of the drug in this homogenous mixture was applied evenly onto the gel surface (Fig. 1A). The glass dish was closed and transferred to an orbital platform shaker, and the rotational speed was set to 55 rpm (Unimax 1010, Heidolph Instruments, Schwabach, Germany). The incubation was performed for 30 min at 37 °C (Hera Cell Incubator, Kendro, Hanau, Germany).

After the incubation time, the gel was washed thoroughly to ensure complete removal of unbound compound and not yet dissolved drug particles. Therefore, a self-designed washing device was used which was lathed from a Teflon® block so that it fit tightly onto the ground joint of the glass dish. The inner part held elaborate milled-out portions to allow efficient washing cycles and yet support the gel sufficiently without compromising its integrity. The gel was washed with approximately 9 mL PBS (Fig. 1B). The

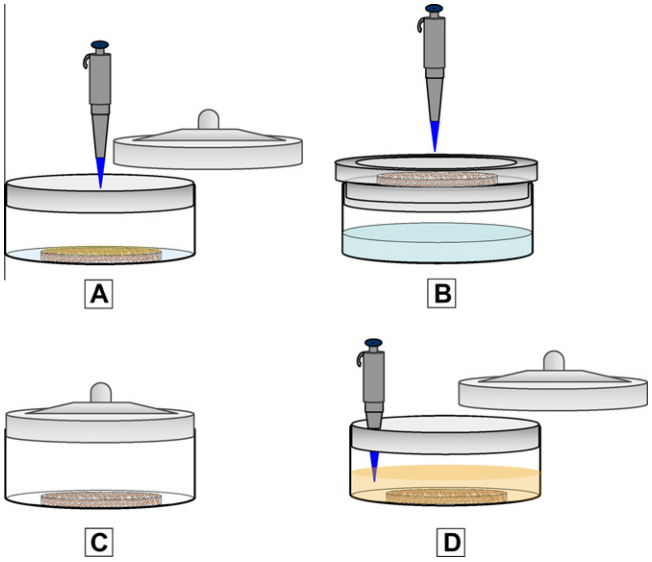

Fig. 1. Schematic illustration of the tissue gel experiments: (A) 500 µL of a mixture of the drug formulation with artificial nasal fluid was applied on the gel surface and incubated for 30 min at 37 °C. (B) Washing procedure with PBS using a custom-made perforated Teflon® support for the gel. (C) Transfer of the washed gel into a fresh ground-joint glass dish. (D) Desorption of drugs from the tissue gel into human plasma and drawing of samples of 1.0 mL while replacing the volume with fresh pre-warmed plasma. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

washed gel was transferred into a fresh glass dish (Fig. 1C). Washing buffer was thoroughly mixed with MeOH (1:1) to dissolve any solid drug particles. For each compound, a gel without embedded tissue was treated accordingly. All samples contained the same proportion of ANF, PBS, and MeOH. Samples were stored at −40 °C until analysis.

2.9. Desorption of drugs from the polyacrylamide-tissue gel

The washed gel was transferred into 10.0 mL human plasma (FP, Bud) or whole blood (AZ) of 37 °C, respectively, and gently shaken with a rocking platform shaker (Mini-rocker, Hartenstein, Würzburg, Germany) for 1 h at 37 °C. Samples of 1.0 mL were drawn after 15, 30, and 60 min (Fig. 1D). The volume withdrawn was replaced with pre-warmed fresh human plasma or whole blood, respectively. Samples were stored at −40 °C until analysis.

2.10. Sample preparation, analysis and HPLC conditions

Samples of adsorption experiments were centrifuged for 30 min at 13,000*g* at 10 °C (Microfuge® 22R Centrifuge, Beckman Coulter, CA, USA). Supernatants were directly injected into the high-performance liquid chromatography (HPLC) system. Typically, 50 µL of sample was injected. Linearity was given from 10 to 500 ng/mL for FP, Bud, and AZ, and coefficients of correlation of the calibration curves were at least $r = 0.99$.

Plasma samples of 1.0 mL (gel desorption) were mixed with 50 µL internal standard solution (amcinonide 5 µg/mL) and extracted twice with 3 mL diethylether for 20 min, using a roller mixer, followed by centrifugation at 800*g* (Eppendorf Centrifuge 5702, Eppendorf, Hamburg, Germany) for 1 min at room temperature. The organic phases were combined and evaporated to dryness under a gentle stream of nitrogen at 25 °C. The resulting residue was reconstituted in methanol.

AZ blood samples of 1.0 mL were mixed with 50 µL internal standard solution (dimetinden maleate 5 µg/mL) followed by

addition of 250 μL NaOH. After two extractions with 4.5 mL hexane/*n*-octanol (95:5) for 20 min, the samples were centrifuged for 5 min at 2000*g* at room temperature. The organic phases were combined, incubated with 150 μL of 0.2% acetic acid, and vortexed for 2 min. The aqueous phase was used for injection into the HPLC system directly. Typically, 20 μL of sample was injected. Linearity was given from 10 to 200 ng/mL for FP and 10–400 ng/mL for Bud and AZ, and coefficients of correlation of the calibration curves were at least $r = 0.99$.

The HPLC system was a Waters HPLC (Milford, USA) consisting of a 1525 binary pump, a 717 plus autosampler, and 2487 dual wavelength absorbance detector. Analysis was performed on a Symmetry C_{18} column (150 × 4.6 mm I.D., 5 μm particle size, Waters, USA) for the glucocorticoids and Lichrospher® 100 CN (250 × 4 mm I.D., 5 μm particle size, Knauer, Germany). Data collection and integration were accomplished using Breeze™ software version 3.4. A flow rate of 1 mL/min was used, and detection wavelength was set to 254 nm for FP and Bud. AZ was analyzed with a flow rate of 0.75 mL/min, and detection wavelength was set to 210 nm. The mobile phase for glucocorticoids consisted of water containing 0.2% (v/v) acetic acid (A) and acetonitrile (B). For FP, the gradient elution started at 60:40 (v/v) A/B increasing linearly to 29:71 (v/v) A/B over 30 min. For Bud, the gradient elution started at 60:40 (v/v) A/B increasing linearly to 40:60 (v/v) A/B over 20 min. The mobile phase for isocratic elution of AZ consisted of a 50:50 composition of water (containing 0.4% phosphoric acid and 0.8% triethylamine) and acetonitrile as described previously [28].

2.11. Inhibition of IL-8 secretion by plasma samples after desorption from polyacrylamide-tissue gel

Samples obtained from gel desorption experiments were also used for cell culture incubations. In case of AZ experiments, new adsorption/desorption experiments were performed and plasma was used instead of whole blood. For all investigated drugs, the plasma was completely replaced with fresh pre-warmed plasma after 1 h incubation period and incubated for another 2 h at 37 °C. All samples were stored at −80 °C until further use in cell culture experiments.

At subconfluence, A549 cells were incubated for 3 h with diluted plasma samples (100 μL plasma in 3 mL medium), thereafter stimulated with LPS (50 μg/mL) for 24 h. Cell culture supernatants were harvested and centrifuged for 10 min at 1000*g* at 20 °C to remove cell debris and stored at −80 °C until further analysis. After each experiment, the number of viable cells was determined after staining with trypan blue. The number of living cells was equivalent after each treatment (overall mean, $1.58 \pm 0.1 \times 10^6$ cells).

The concentrations of IL-8 secreted into the cell culture medium from the epithelial cells were analyzed using a commercial enzyme-linked immunosorbent assay (ELISA) test kit (Enzo Life Sciences, Lörrach, Germany) according to the manufacturer's protocol. The levels of IL-8 were assayed at an optical density of 450 nm using ELISA reader Multiscan Accent; Thermo, Vantua, Finland.

3. Results

3.1. Establishment of the pharmacokinetic model

Human respiratory tissue pieces were embedded into an inert matrix. A polyacrylamide gel revealed to offer the best properties for this purpose. The tissue gel was solid enough to be handled in subsequent washing and incubation steps without losing its integrity (Fig. 1). Drug doses of 9 μg from commercially available nasal sprays and artificial nasal fluid were loaded onto the gel surface of approximately 11 cm^2 and incubated for 30 min, which would be the maximum contact time of drug particles on the nasal mucosa before mucociliary clearance [21]. The gel surface was washed extensively to remove all nonadsorbed compounds and undissolved drug particles.

In contrast to the previous experimental settings, the new model allowed to use commercially available nasal sprays containing drug suspensions and to account for the mucociliary clearance. In a proof-of-concept study, the binding of fluticasone propionate (FP) and budesonide (Bud) to human respiratory tissue was determined and compared to the previous results (Fig. 2). In the simple tissue experiments with drug solutions [18], the relation of concentrations in the drug-loaded tissue were 1:0.36 for FP and Bud, respectively, at $t = 0$ (before incubation with human plasma) and 1:0.39 at $t = 60$ min. In contrast, in the new model using a tissue gel and drug suspensions, the concentrations in tissue were 1.77 ± 0.02 ng/mg for FP and 1.89 ± 0.32 ng/mg for Bud at $t = 0$. After 60 min equilibration with human plasma, the drug concentrations retained in the tissue were 1.09 ± 0.03 ng/mg for FP and 0.08 ± 0.18 ng/mg for Bud. Thus, the relation of concentrations in the tissue were 1:1.07 for FP and Bud, respectively, at $t = 0$ (before incubation with human plasma) and 1:0.07 at $t = 60$ min.

3.2. Respiratory tissue binding and retention of intranasal glucocorticoids and an antihistamine

The tissue binding properties of another compound class were determined in comparison with the two topical glucocorticoids FP and Bud. Therefore, a commercially available nasal spray containing the antihistamine azelastine hydrochloride (AZ) as solution was incubated with the tissue gels (Fig. 3). Compared to the glucocorticoids, significantly higher concentrations of AZ were bound to the tissue after 30 min incubation with the tissue gel ($t = 0$). At this time point, 7.99 ± 0.84 ng/mg of AZ was bound to the tissue whereas less than 2 ng/mg of FP or Bud was bound. After equilibration of the tissues gels with human plasma, tissue concentrations of Bud decreased most pronounced from 1.89 ± 0.32 ng/mg to 0.26 ± 0.18 ng/mg (15 min) and from 0.04 ± 0.31 ng/mg (30 min) to 0.08 ± 0.18 ng/mg (60 min). In contrast, both FP and AZ were retained to higher degree in the tissue. Tissue levels of FP decreased

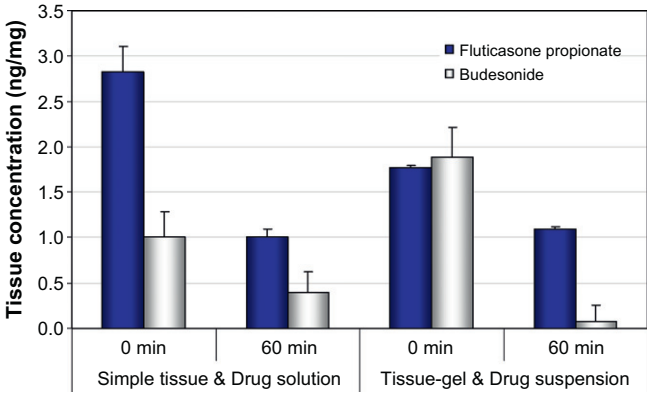

Fig. 2. Comparison of concentrations of fluticasone propionate and budesonide in human respiratory tissue in two different experimental approaches. We previously [18] worked with a simple tissue binding setting where the glucocorticoids were applied as solutions (left side of the diagram). The new model system employs tissue gels, and the drugs are applied as suspensions from commercially available nasal sprays (right side of the diagram). Tissue concentrations were determined before incubation in human plasma (0 min) and after 60 min incubation in human plasma at 37 °C. The columns represent the mean and mean deviation of the mean of three independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

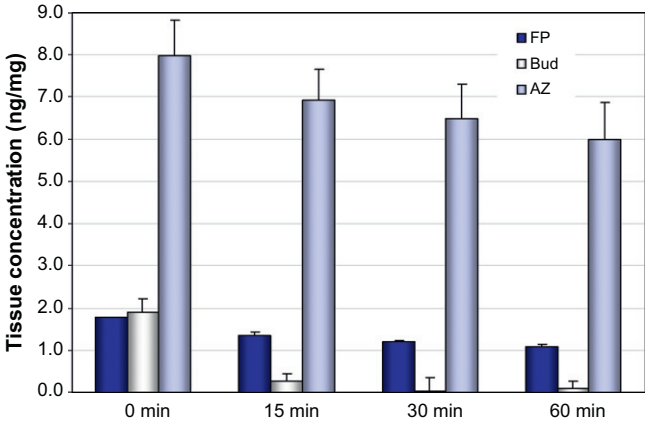

Fig. 3. Comparison of concentrations of fluticasone propionate (FP), budesonide (Bud), and azelastine (AZ) in human respiratory tissue. The left columns represent the glucocorticoid concentration in charged tissue before incubation in human plasma. Tissue concentrations were determined before incubation in human plasma (0 min) and after 15, 30, and 60 min incubation in human plasma at 37 °C. The columns represent the mean and mean deviation of the mean of three independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

from 1.77 ± 0.02 ng/mg to 1.35 ± 0.09 ng/mg (15 min) and from 1.21 ± 0.02 ng/mg (30 min) to 1.09 ± 0.03 ng/mg (60 min), whereas tissue concentrations of AZ decreased from 7.99 ± 0.84 ng/mg to 6.92 ± 0.74 ng/mg (15 min) and from 6.47 ± 0.84 ng/mg (30 min) to 5.98 ± 0.89 ng/mg (60 min).

These concentrations reflect the binding exclusively to the respiratory tissue. For each experiment, a control was run with a tissue-free gel to determine the binding to the matrix itself. Tissue binding to the tissue-free gel matrix was subtracted from the binding to the tissue gel. In every experiment, the binding to the tissue gel was above the binding to the tissue-free gel. However, binding of the drugs to the gel matrix differed (Fig. 4). Whereas only 23 ± 5% and 33 ± 2% of the bound FP and AZ amounts, respectively, accounted for binding to the gel matrix, 80 ± 1% of the binding of Bud was to the gel matrix. Interestingly, despite this high background binding of Bud, there was a statistically significant correlation between the drug concentrations retained in the tissue after 60 min and the apparent volumes of distribution of the respective drugs (Fig. 5; $r = 0.9999$, $p \leqslant 0.05$; two-sided test).

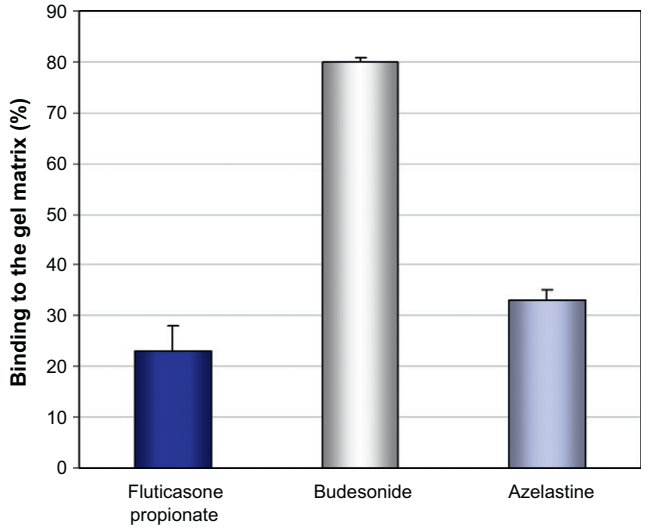

Fig. 4. Nonspecific binding of fluticasone propionate, budesonide, and azelastine to the polyacrylamide gel matrix. The columns represent the mean and mean deviation of the mean of three independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Anti-inflammatory effect of tissue-retained drug fractions: inhibition of IL-8 secretion

Since anti-allergic effects are dependent on the availability of the drug in the respiratory tissue and the drugs' effects on inflammatory parameters, we determined the effects of tissue-bound FP and AZ on the inhibition of IL-8 secretion from LPS-stimulated A549 cells (Fig. 6). Therefore, an aliquot of the plasma containing drugs desorbed from the tissue gel (Gel with tissue) and the tissue free, but compound-exposed polyacrylamide gel (Gel w/o tissue) was incubated with the A549 cells. Both the plasma equilibrated with the tissue gel and the plasma exposed to the compound-exposed matrix decreased IL-8 secretion versus control (plasma incubated with drug-free gel without tissue). However, the inhibition of IL-8 release from A549 cells was more pronounced for the tissue gels. FP released into plasma from the tissue gel resulted in 56 ± 3 pg/mL, whereas the drug bound to the gel matrix only decreased IL-8 only to levels of 91 ± 3 pg/mL. IL-8 release from A549 cells was less influenced by AZ. AZ released into plasma from the tissue gel resulted in 306 ± 11 pg/mL IL-8, whereas the drug bound to the gel matrix had no influence on IL-8 concentrations and was even slightly higher than control concentrations (380 ± 1 pg/mL versus 338 ± 12 pg/mL for control). LPS-stimulated A549 cells secreted 470 ± 6 pg/mL IL-8 when they were incubated with plasma not previously exposed to a polyacrylamide gel (data not shown).

4. Discussion

In the present study, we developed a novel model system for comparative evaluation of intranasal pharmacokinetics of commercially available drug preparations that accounts for particle dissolution in nasal fluid, mucociliary clearance, diffusion, and

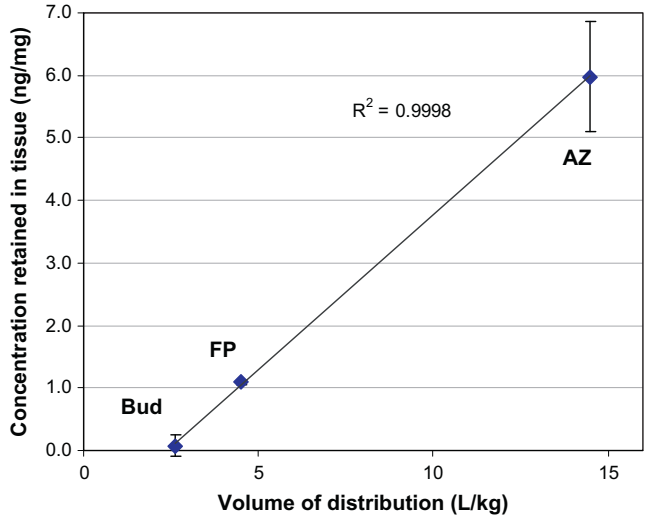

Fig. 5. Correlation of the tissue concentrations of fluticasone propionate (FP), budesonide (Bud), and azelastine (AZ) retained after 60 min equilibrium in human plasma at 37 °C with the apparent volume of distribution of these compounds [35,49,50]. Data points of tissue concentrations represent the mean and mean deviation of the mean of three independent experiments. The coefficient of correlation was $r = 0.9999$ ($p \leqslant 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

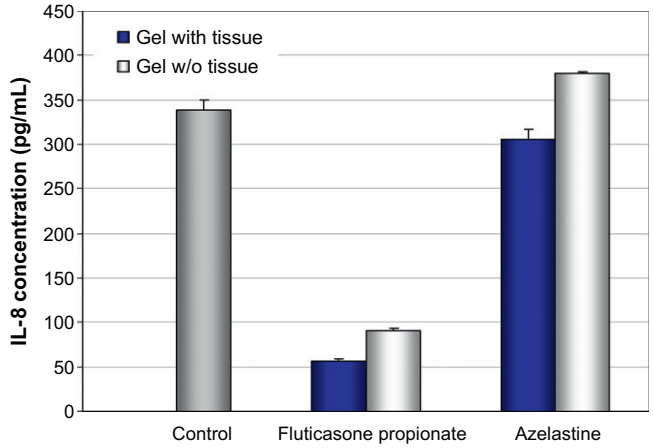

Fig. 6. Inhibition of IL-8 secretion from LPS-stimulated A549 cells incubated with plasma which was before exposed to drug-loaded gels over 60 min. The drugs desorbed into plasma from the polyacrylamide-tissue gel (Gel with tissue) and the tissue free, but compound-exposed polyacrylamide gel (Gel w/o tissue) decreased IL-8 secretion versus control (plasma incubated with drug-free gel without tissue). The columns represent the mean and mean deviation of the mean of three (FP, AZ), respectively, and four (control) independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

binding to tissue as well as tissue retention. Furthermore, this model can be linked with pharmacodynamic assays to determine the effects of the tissue-bound drug fraction.

Regarding fluticasone propionate (FP) and budesonide (Bud) as model compounds, the results obtained with the tissue gel system revealed distinct differences compared to the previous simple tissue binding experiments. Though absolute bound or retained concentrations cannot be directly compared between experiments since they show high variability between tissue batches, the relation of bound or retained fractions of FP and Bud are suitable measures for comparison.

In the simple tissue binding experiments, these relations were relatively consistent. We recently found binding relations of drug-loaded human nasal tissue of 1:0.36 for FP and Bud, respectively, and 1:0.39 after 1 h equilibration of tissue in human plasma [18]. In previous experiments with human nasal tissue, we determined binding relations of 1:0.39 after saturation of the tissue with FP and Bud, respectively, and 1:0.49 after drug desorption [17]. Binding experiments with human lung tissue revealed similar results with binding relations of drug-loaded tissue of 1:0.35 for FP and Bud, respectively, and 1:0.32 after equilibration of tissue in human plasma [14]. Thus, roughly three times higher concentrations of FP were bound as well as retained in the respiratory tissues compared to Bud. In these experimental settings, both glucocorticoids were applied as solutions so that factors such as dissolution rates played no role.

Using respiratory tissue embedded into a solid matrix to resemble a continuous surface, FP and Bud were bound to the same extent to the tissue gel with binding relations of 1:1.07. This indicates that the present model realistically mirrors physiologic conditions *in vivo* where solubility and tissue absorption of intranasally applied drugs compete with mucociliary clearance mechanisms. Mucociliary clearance may remove the drug from the respiratory region in the nasal cavity before it has had a chance to dissolve and diffuse into the mucosa [3,21,22]. We recently showed that glucocorticoid concentrations in the aqueous supernatants of commercially available drug suspensions for intranasal application and the drugs' solubility in artificial nasal fluid were very different for FP and Bud [18]. The higher already dissolved fraction of Bud is instantaneously available for diffusion and binding processes, and its higher solubility in nasal fluid increases the chance of binding of a larger drug fraction to the nasal tissue before removal by mucociliary clearance. In contrast, although FP has a higher tissue binding potential than Bud [17,18,29], its minor already dissolved fraction in the nasal spray and its slower dissolution in nasal fluid subject any undissolved drug particles to mucociliary clearance. In the present study, we thoroughly washed the tissue gel surface after 30 min incubation to remove any drug that did not yet diffuse into the tissue gel. We thus mimicked the mucociliary clearance since it has been reported that a normal mucociliary transit time in humans is not longer than 30 min [21]. Based on our results with the tissue gel, we conclude that *in vivo* after application of nasal spray suspensions equal concentrations of FP and Bud might be bound to the nasal tissue.

Besides the glucocorticoids, we elucidated the tissue binding of a representative of another drug class used for the treatment of allergic rhinitis. The antihistamine azelastine (AZ) displayed a more 4-fold higher binding to human respiratory tissue compared to FP or Bud. In contrast to FP and Bud suspension nasal sprays, AZ is administered as aqueous solution. Thus, the compound is instantaneously available for diffusion and binding to the tissue and less prone to mucociliary clearance, resulting in high tissue concentrations.

After initial binding to tissues, drugs are retained in the tissue for various lengths of time. A prolonged tissue retention is a desired characteristic for locally effective drugs [2,30]. In earlier tissue binding experiments, we determined an approximately 3-fold higher retention of FP compared to Bud in respiratory tissue [14,17,18]. In the tissue gel experimental setting, we now observed a retention of 1:0.07 for FP and Bud, respectively. These roughly 10-fold higher tissue concentrations of FP compared to Bud are consistent with the high systemic availability of Bud after intranasal administration [31], while negligible systemic concentrations are observed for FP [32]. On the other hand, the tissue binding data of FP and Bud obtained with our model contrast to the results of a previous *in vivo* study, showing that Bud was longer retained in nasal mucosa biopsies than FP. The intracellular formation of fatty acid esters of Bud was identified as basis for the prolonged tissue retention of Bud [33]. Since it is unlikely that the tissue used in our model was viable enough to allow the formation of budesonide esters, the tissue retention of Bud might be slightly underestimated.

Compared to the glucocorticoids, even higher concentrations of the antihistamine AZ were retained in the respiratory tissue. To our knowledge, no investigations regarding binding behavior of antihistamines to respiratory tissues have been performed yet. After 60 min equilibrium with human plasma, approximately 5-fold higher tissue concentrations of AZ compared to FP and 77-fold higher levels compared to Bud were determined. This tissue retention revealed an excellent correlation with the apparent volume of distribution of the respective drugs ($r = 0.9999$, $p \leqslant 0.05$). The apparent volume of distribution (V_d) reflects the proportionality constant of the administered drug dose in relation to its plasma concentration. Thus, drugs with pronounced distribution into organs and tissues typically have high volumes of distribution. Consistent with this consideration, we see the lowest V_d for Bud and highest for AZ. Though we only analyzed three different drugs, the notably good correlation of drugs' binding behavior determined with our model and the drugs' V_d is suggestive of the validity and physiologic proximity of our model system.

Typically, drugs with low tissue retention are rapidly absorbed into systemic circulation. Measures for the drugs' absorption and distribution into systemic circulation are maximal plasma levels (c_{max}), the time of maximal plasma concentrations (t_{max}), and the area under the curve (AUC). After intranasal administration, a t_{max} of 30 min was observed for Bud [31], while t_{max} values of 55 min and 120–180 min were found for FP and AZ, respectively [34,35].

Very similar results were obtained after inhalation of FP and Bud [36]. Again, there is a significant correlation of the tissue retention concentrations obtained with our model and the t_{max} values of Bud, FP, and AZ after intranasal application determined in a clinical setting ($r = 0.9993$; $p \leqslant 0.05$).

Sufficiently high drug concentrations at the therapeutic target site are the prerequisite for clinical effectiveness, and the potency of the drug governs the magnitude of the effect. Various inflammatory mediators play a role in the early and late phase allergic rhinitis [37,38]. We elucidated the inhibition of IL-8 secretion from the human epithelial A549 cells as an example to demonstrate that our model allows the investigation of pharmacodynamic effects of the tissue/matrix bound drugs. FP was known to inhibit IL-8 release from human epithelial cells [39], and AZ has been also shown to decrease IL-8 secretion from various cells [40,41]. Despite the 4-fold higher initial tissue binding of AZ compared with FP, the inhibitory effect of FP on IL-8 release was approximately 5-fold more pronounced compared with AZ. This result is consistent with the clinical observation that intranasal glucocorticoids produce a greater relieve of nasal symptoms in allergic rhinitis [42]. However, since IL-8 is only one example of an inflammatory mediator, we do not claim that our results give a comprehensive explanation for the different efficacies of glucocorticoids and antihistamines in allergic rhinitis. Interestingly, recent combinations of intranasal FP and AZ have been shown to produce additive clinical benefit compared to either drug alone [43,44]. Especially, the rapid onset of action of AZ has been discussed as advantage [45]. From our model, we can conclude that the faster onset of action of AZ is also based on its fast and pronounced diffusion into respiratory tissue since it is administered as solution.

Our model has some limitations as well. Though the tissue gel was coated with artificial nasal fluid, no epithelial cell monolayer covered its top side. The rational was that we intended to keep the model simple enough to allow vigorous washing of the surface after the incubation without losing drug-loaded cells during this process. It cannot be excluded that an additional epithelial cell monolayer altered the kinetics of drug absorption into the underlying tissue. Yet, the mucosa represents a rather weak diffusion barrier, readily allowing penetration of molecules up to 1000 Da [46]. It has been shown that the permeability of anti-allergic drugs across human nasal epithelial monolayers was highly correlated with the drugs' lipophilicities [47]. Additionally, the drug formulation plays a crucial role for the permeability [48]. Thus, the tissue concentrations we measured were the result of drug dissolution, uptake, and retention in the human tissue. A clear limitation of our model is that is does not allow to determine permeability coefficients or study the role of drug transporters. For the elucidation of those parameters, cell culture models are most qualified, preferentially if using human nasal epithelial cells. Eventually, mucociliary clearance is a dynamic process *in vivo* while we completely removed the drug formulations after 30 min contact time. Thus, the simulation of the clearance process in our model represents only an attempt of adaptation.

To summarize, we developed a model system for comparative analysis of pharmacokinetics and pharmacodynamics of the intranasally administered glucocorticoids FP and Bud and the antihistamine AZ. The model system employs respiratory tissue embedded into a solid matrix, which allows simulation of particle dissolution processes on the nasal mucosa and mucociliary clearance mechanisms. We demonstrated that under these conditions a glucocorticoid with a rather high aqueous solubility, but low tissue binding affinity (Bud), was initially equally well bound to respiratory tissue as a corticosteroid with very low water solubility and high tissue binding (FP). Drugs administered as solution such as AZ are highly bound to tissue. Besides determination of the tissue binding and retention characteristics, the model allows comparative elucidation of the pharmacodynamic effects of the tissue-bound drugs. Thus, we suggest that our model is suitable for comparative characterization of the intranasal pharmacokinetics and pharmacodynamics of different drugs or drug preparations.

Conflict of interest

DB declares no conflict of interest.

CB has received consultant or speakers honorary and/or research grants from Schering-Plough, GlaxoSmithKline, Uriach, UCB, and Procter & Gamble within the last 5 years.

PH has received consultant or speakers honorary and/or research grants from Altana/Nycomed, GlaxoSmithKline, and Schering-Plough within the last 5 years.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Acknowledgement

We would like to thank Dr. Kardziev from the Thoraxzentrum Bezirk Unterfranken for providing us with human lung tissue.

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