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Short communication

Presence of fluticasone propionate on human nasal mucosal surface and in human nasal tissue over a period of 24 h after intranasal application

Background: Once-daily use of nasally applied glucocorticoids was demonstrated to be effective in the treatment of allergic rhinitis. The aim of the study was to measure concentrations of fluticasone propionate (FP) in nasal secretion and nasal tissue over a period of 1 day after a single application of 100 μg FP.
Methods: Twenty-six patients applied nasal FP spray at different time intervals before surgery. Cotton swabs, used to clean the mucosal surface, and resected nasal tissue were extracted. FP concentrations were determined by RIA.
Results: FP was found in nasal secretions in concentrations from 15 to 1 μg/g over a period of 20 h, and in nasal tissue in concentrations from 200 to 13 ng/g up to 24 h after the single application.
Conclusions: The long-persisting high concentrations of FP provide the pharmacokinetic basis for once-daily treatment.

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Key words: fluticasone propionate; human nasal tissue; nasal secretion; tissue binding.

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Fluticasone propionate (FP) is a steroid derivative with potent topical anti-inflammatory activity and minimal systemic effects when applied intranasally.

The aim of this study was to investigate the pharmacokinetics of a glucocorticoid after nasal application. This may provide a rational basis for the use of a once-daily dosage regimen of the drugs. Clinical experience has demonstrated the efficacy of once-daily treatment; however, that experience has no pharmacokinetic background. So far, no data are available on the local tissue concentrations of glucocorticoids after topical application.

FP was chosen for this study because of its negligible systemic concentrations after nasal application (1), and because a highly sensitive assay was available.

The dissolution of FP crystals contained in the aqueous nasal spray is a slow process. In the application of FP aerosol into the lung, the dissolution of the crystals was the rate-limiting step for the delivery of the drug to the lung (2).

Experiments with human nasal tissue *in vitro* (3) have shown that dissolved glucocorticoids are bound to the tissue within a relatively short time. Equilibrium between dissolved substance and tissue-bound glucocorticoids was reached within 30 min. The more lipophilic glucocorticoids FP and beclomethasone-17-monopropionate (17-BMP) were bound to a greater extent than budesonide and flunisolide. Glucocorticoids have a considerable affinity to nasal tissue. Even after equilibration of tissue saturated with corticosteroids with fresh human plasma, we found high concentrations remaining within the tissue. These concentrations were higher for FP and 17-BMP than for budesonide and flunisolide. The glucocorticoids bound to tissue elements form a kind of depot for binding to the glucocorticoid receptors because of the exchange between tissue-bound and receptor-bound molecules.

As in *in vitro* experiments with human lung tissue (4), FP was bound *in vivo* in lung tissue in higher amounts than budesonide and persisted in the tissue for a period of 15 h (2).

The affinities of the glucocorticoid molecules to the receptor, regulating their pharmacodynamic action, are higher for FP and the active metabolite of BDP,

beclomethasone-17-propionate. Budesonide and flunisolide have lower affinities (5). Therefore, the more lipophilic glucocorticoids may exhibit a greater clinical efficacy after inhalation (6).

The local pharmacodynamics of FP after its nasal application as an aqueous solution are determined by the following steps:

1) delivery of FP from the nasal spray into the nasal secretion
2) diffusion into and binding to the nasal tissue
3) diffusion within the tissue and binding to the glucocorticoid receptors
4) local metabolism within the nasal tissue
5) delivery from nasal tissue into the circulation with subsequent dilution in the bloodstream and metabolism in the liver.

The present investigation was designed to measure the time course of the disappearance of FP from the surface of the nasal mucosa. Simultaneously, the monitoring of FP concentrations in the nasal tissue should answer the question of the residence time of FP inside the nasal tissue. The measurements had to be done on material delivered from different patients undergoing resections of nasal tissue at varying time intervals after nasal application of FP.

Material and methods

Patients

Tissue samples were obtained from 26 patients, 16 male, 10 female, aged 26–74 years, from centers in Ghent and Münster, who had to undergo septoplasty, septorhinoplasty, or functional endoscopic sinus surgery. The diagnoses were chronic sinusitis (5), nasal polyposis (18), inverted papilloma (2), and nasal carcinoma (1). Approval of the ethics committees of the University Hospital Ghent and the University of Münster was obtained. Patients were informed of all possible risks of the study and gave written informed consent prior to treatment. Patients with obstruction of their nostrils, or who were taking steroid medications or other research medications, as well as pregnant or lactating women, were excluded from participation in this investigation. All other medications could be continued, provided that their use did not affect nasal function.

Each patient applied 2×50 µg FP as an aqueous nasal spray in each nostril once at a given time prior to operation. Patients were instructed in the use and function of the nasal FP spray.

During nasal surgery, samples were taken from each patient's nose, and the interval between nasal application of FP and surgery was documented. Nasal secretion of the mucosal surface was collected with cotton pads. Nasal tissue from the inferior turbinates, which was removed during surgery, was collected and deep frozen until analysis.

Quantification of FP in nasal swabs

The cotton pads were placed in plastic tubes (Greiner no. 421 161) and extracted with ether to remove any contamination residues, dried, and weighed. After collection of nasal secretion by vigorous cleaning of the nasal mucosa, the cotton pad was replaced in the plastic tube and weighed again to determine swab weight. The cotton pads were extracted two times with 4 ml and thereafter with 2 ml methanol by vortexing for 10-s and 20-min extractions in rotating bottles. The extracts were evaporated to dryness under nitrogen. The residue was dissolved in 200 µl of a mixture of equal parts of ethanolic tricine buffer and gelatin containing tricine buffer. The content of FP was determined by radioimmunoassay (RIA), as described earlier (2).

Quantification of FP in nasal tissue

Deep-frozen nasal tissue was weighed, minced, and homogenized after addition of a twofold quantity of water on ice with an ultraturrax. The ultraturrax was washed three times with 1 ml water. Tissue suspension and washing suspensions were combined and freeze-dried. The dried residue was weighed. An amount of 0.1 g of the powdered, freeze-dried nasal tissue was extracted two times with 5 ml methanol. The combined methanol extracts were stored at $-20°C$ until analysis for FP by RIA. Before the assay, aliquots of the methanolic extracts were evaporated to dryness under nitrogen, and the residue was dissolved in equal parts of ethanolic tricine buffer and gelatin containing tricine buffer.

Radioimmunoassay (RIA)

The RIA was performed by mixing 200 µl extract or calibration solution with 100 µl FP-antibody solution (Glaxo-Wellcome, London, UK). After incubation at 0–4°C for 20 min ^{3}H-labeled FP solution (Glaxo-Wellcome) was added and incubated for 20 min at 0–4°C. A 1-ml suspension of 0.25% charcoal coated with 10% dextran T70 (Sigma) was added and incubated after mixing for 10 min at 0–4°C. After centrifugation (3300 rpm at 4°C), 1 ml supernatant was mixed with 3 ml of the scintillation cocktail Aqua Safe 500 Plus (Zinnser Analytik, Frankfurt/Main), and radioactivity was measured in a scintillation counter Rackbeta 1219 (Zinnser Analytik).

Calibration solutions contained 0.03-3 ng/ml FP in equal parts of ethanolic tricine buffer and gelatin containing tricine buffer. The concentrations of FP were calculated by the linear regression of dpm without FP divided by the decay per minute (dpm) of the solutions with known concentrations of FP against the log of the concentrations of the calibration solutions.

Results

The content on FP in the nasal secretions, analyzed by RIA, was calculated per gram nasal secretion. FP concentrations in the nasal secretion varied widely between different patients; however, there was a steady decline from 9–14 µg/g in the first 2 h to 1 µg/g at 20 h after application (Fig. 1). All samples contained detectable amounts of FP; even after 22–24 h, 0.1–0.3 µg/g FP could be found. The lower limit of quantification was 30 ng/g.

Tissue concentrations were calculated for the FP content of the fresh tissue. FP concentrations rose fast after application; the maximum absorption could be estimated at 1.5 h (Fig. 2). Because no tissue was obtained within the first hour after application, the absorption phase could not be monitored. FP concentrations declined from 200–100 ng/g over a period of 24 h to concentrations of 18–13 ng/g in an exponential manner. The lower limit of quantification was 10 ng/g.

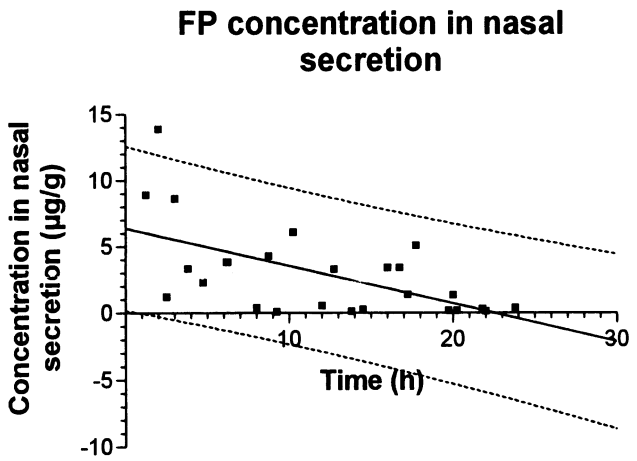

Figure 1. FP concentrations in nasal secretions at different time intervals after intranasal administration of 100 µg FP. Each data point represents concentration in secretion from single patient. Dotted lines indicate 95% prediction interval of FP concentration in nasal secretion.

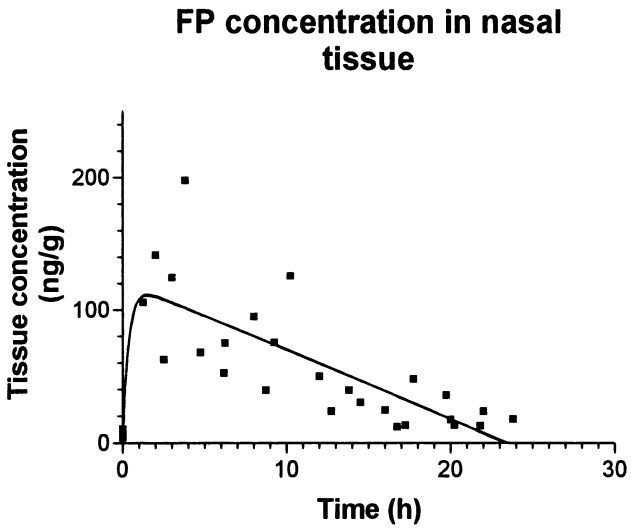

Figure 2. FP concentrations in human nasal tissue at different time intervals after intranasal administration of 100 µg FP. Each data point represents concentration in tissue from single patient.

Discussion

We report for the first time concentrations of FP in nasal secretion and nasal tissue at different times after nasal application. The concentrations of FP on the surface of the nasal mucosa remained relatively high (µg/g) over a period of about 20 h compared to the FP content of the nasal tissue (ng/g). Therefore, the reason for the long residence time of FP in the nasal tissue was obviously the high concentration of FP on the mucosal surface, which persisted for a long time due to the extremely low water solubility of FP. The slow dissolution of the FP crystals was the basis for a slow release into the nasal secretions. The FP crystals remaining on the surface of the nasal mucosa formed a deposit of the drug, leading to a linear release of FP over a period of 1 day.

The dissolved, highly lipophilic FP was absorbed very rapidly, so that the absorption phase could not be observed within the first hour. The time course of the decline of FP tissue concentration followed a one-compartment pharmacokinetic model, with a terminal half-life of about 12 h. Thus, the long residence time of FP within the tissue is the argument for a once-daily application of FP, which provides an efficient anti-inflammatory and antiallergic effect for 24 h.

It is interesting to compare the concentrations of FP found in nasal tissue and in lung tissue. After inhalation of 1000 µg FP, the highest concentrations found in central lung tissue were 15–22 ng/g; in peripheral lung tissue, the highest concentration was 5 ng/g (1). In contrast, the concentrations in nasal tissue after application of 100 µg per nostril were 100–200 ng/g. These differences reflected the large differences in the surface of the target organs, leading to high concentrations in nasal tissue, as a relatively large dose was applied to a very small surface. The relatively high dose of 100 µg glucocorticoid given via the nasal route led to 10 times higher tissue concentrations than in lung tissue. There is no doubt that inhalation of 1000 µg produces a sufficiently high anti-inflammatory effect in the lung. The resulting FP concentrations in lung tissue necessary for a therapeutic effect obviously are 10 times lower than the concentrations of FP in nasal tissue obtained in our investigation.

It therefore is justified to suggest that doses given for treatment of nasal allergies are higher than necessary. It should be possible to achieve the desired therapeutic effect with a lower dose. A consequence of the high drug concentration in nasal tissue is that clinical differentiation between the different glucocorticoids is hardly possible.

That explains why differences in the efficacy of glucocorticoids cannot be seen in clinical studies after nasal application, despite the differing intrinsic activities of the various glucocorticoids (5) and the clear rank order of the glucocorticoids after inhalation (6).

Regarding systemic adsorption, the equilibration of tissue concentration with plasma leads to very low plasma concentrations near the limit of detection even of a very sensitive RIA, due to the very small size of the organ relative to the total volume of circulating blood. In two separate studies, the systemic adsorption of intranasal FP was investigated after

1) single doses of 200 µg under normal conditions and in allergen-induced rhinitis
2) a single 800-µg dose and twice daily dosing with 200 µg for 5½ days (1).

In the first study, plasma FP concentrations remained below the lower limit of quantification (LLQ) of the assay (50 pg/ml), even under the condition of nasal mucosal inflammation associated with allergen-induced rhinitis

(1). After twice daily dosing with 200 μg, occasionally detectable concentrations of FP (<100 pg/ml) were observed in only half the subjects. Even at four times (800 μg) the maximum recommended single dose, most of the FP plasma concentrations were below the LLQ of the assay (1). In comparison of plasma concentrations after 800 μg intranasal and 2 mg i.v. doses of FP, the bioavailability of intranasal FP was estimated at about 1.8%. It seems that after oral application of 800 μg FP plasma FP concentrations would not be detectable; therefore, the very small quantity of FP present in the systemic circulation after intranasal administration is likely to represent the fraction absorbed from the nasal mucosa. In conclusion, systemic exposure caused by intranasal FP occurs only to a very small extent (1).

These very low plasma concentrations are unlikely to lead to systemic side-effects. The long residence time of FP within the target tissue does not lead to a long presence of effective concentrations in plasma. Consequently, the application of 200–336 μg of different glucocorticoids for 4 days did not cause a suppression of urinary cortisol excretion normalized for creatinine or morning serum cortisol nor affect the response to ACTH (7).

In conclusion, the nasal application of FP leads to high and long-persisting drug concentrations within the target organ, justifying a once-daily dosing regimen. The slow release to the systemic circulation explains the topical efficacy and the lack of systemic side-effects.

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