Rhinology, 39, 13-16, 2001

The effect of intranasal budesonide spray on mucosal blood flow measured with Laser Doppler flowmetry*

Anders Cervin[1], Anders Åkerlund[2], Lennart Greiff[1], Morgan Andersson[1]

[1] Department of Oto-Rhino-Laryngology, Head and Neck Surgery, University of Lund, S-221 85 Lund, Sweden
[2] AstraZeneca R&D, Lund, Sweden

SUMMARY

Background: Recent reports have shown that, although rare, findings of mucosal ulcers and perforations of the nasal septum in some cases may be associated with the use of topical nasal glucocorticosteroids (GCS). It can been speculated that, a reduction in septal mucosal blood flow causing ischemia may eventually induce septal perforations.
Aim: To evaluate whether a single dose of a potent nasal GCS given in a clinically recommended dose may acutely reduce the mucosal blood flow on the nasal septum.
Methods: Six healthy subjects received in a randomised double blind placebo controlled cross-over procedure one dose of 64 μg budesonide® aqueous nasal spray (Rhinocort aqua®, AstraZeneca R&D, Lund, Sweden) and placebo. One dose was delivered into each nasal cavity by means of a pump spray. As a positive control 140 μg of xylometazoline (Nezeril®, AstraZeneca R&D, Lund, Sweden) was sprayed in the same way, but in an open fashion. A wash-out period of at least 3 days followed each session. Blood flow was measured on the nasal septum with Laser Doppler flowmetry up to 20 min after administration.
Results: Budesonide did not affect the nasal septal mucosal blood flow as compared to placebo, but xylometazoline reduced the septal mucosal blood flow by 60.9±7.1% measured from baseline values.
Conclusion: A single dose of intranasal budesonide aqueous nasal spray has no acute effects on nasal septal mucosal blood flow.

Key words: blood flow, budesonide, Laser Doppler flowmetry, nasal mucosa, xylometazoline

INTRODUCTION

We have recently reported that, although rare, findings of mucosal ulcers and perforations of the nasal septum in some cases may be associated with the use of topical nasal glucocorticosteroids (GCS) (Cervin et al., 1998). If this is a side-effect of nasal GCS it can been speculated that a reduction in septal mucosal blood flow can cause ischemia potentially harmful to the most vulnerable part of the nasal mucosa, i.e., the septum.

Other reports have also shown a rapid onset of effects of GCS which may be attributed to vascular effects. One such investigation has demonstrated that topical budesonide increases nasal inspiratory flow (Day et al., 2000), and reduces mucosal output of cytokines like GM-CSF and IL-5 already after three hours (Linden et al., 2000). These rapid effects cannot be explained by GCS receptor-agonism induced responses via translation and protein synthesis, which may take between six and twelve hours. Thus, acute effects of GCS may be attributed to vasoconstriction resulting in a reduced nasal mucosal blood flow and decongestion.

The potency of GCS can be assessed in the skin by their ability to cause skin blanching, the so called McKenzie test (McKenzie, 1962). The skin blanching effect has been suggested to be caused by vasoconstriction (Sommer et al., 1998). Previous studies have however failed to demonstrate vasoconstriction after topical application of GCS on the nasal mucosa. In studies using the 133Xenon wash-out technique, to measure blood

* Received for publication April 26, 2000: accepted September 6, 2000

flow, budesonide, a potent glucocorticosteroid failed to reduce the total mucosal blood flow (Bende et al., 1983). ^{133}Xenon wash-out, however measures other aspects of the mucosal blood flow than Laser Doppler flowmetry (LDF) (Olsson, 1986). It is believed that LDF measures flow in more superficial parts of the vasculature than the ^{133}Xenon wash-out method. Furthermore, the immediate effects on blood flow just after delivery of the glucocorticosteroid cannot be studied with the ^{133}Xenon wash-out technique.

In the light of the previous studies there seems to be a need for more information whether topical nasal GCS influence nasal mucosal bloodflow. The aim of the present investigation was therefore to study the immediate effects of intranasal budesonide spray on the nasal septal mucosal blood flow measured non-invasively with Laser Doppler flowmetry.

MATERIAL AND METHODS

Study design

A randomised, double blind, placebo controlled, cross over study with respect to budesonide, and an open positive control sequence with respect to xylometazoline.

Subjects

Six healthy volunteers, 4 females and 2 male subjects, participated. Mean age was 26 years (23-41 years). They were evaluated by medical history and were excluded if they had any history of atopic disease, chronic sinusitis, perennial rhinitis, asthma or previous surgery of the nose. The mucosa was normal as seen at anterior rhinoscopy. All stated that they had not suffered from respiratory infections within the past month. No medication was allowed except for study drugs and oral contraceptives. The study design was approved by the Ethics Committee of the Medical Faculty of the University of Lund, Sweden.

Measurements of nasal mucosal blood flow

Nasal mucosal blood flow was measured using Laser Doppler flowmetry (LDF) (Periflux Pf2b, Perimed, Sweden). The method has been described by Tenland (Tenland, 1982), and has been applied in studies of the human nasal mucosa by Olsson (Olsson et al., 1986; Olsson, 1985). It is a non-invasive method where reflected Laser light from the nasal mucosa is analysed and provides microcirculatory parameters such as the concentration of moving blood cells and their velocity. The product of the two is called flux and means to assess the blood flow. The signal is registered in arbitrary units making it suitable for continuous recordings of relative changes, but not for repeated measurements. The probe had an angled tip of 90° and a fiber separation of 0.25 mm (PF 310S, Perimed, Sweden). The experiments were performed with the subject in the sitting position with the chin and forehead resting against a rigid frame. The probe was mounted on a stand, adjustable in all directions

Table 1. The raw data for area under the curve (AUC) for a 20 minute registration period, expressed as percentages.

	Placebo	**Budesonide**	**Xylometazoline**
Subject 1	-825	-11294	7140
Subject 2	-5043	-3326	-26695
Subject 3	-4539	-19520	-34450
Subject 4	-17060	-3771	-42037
Subject 5	-1518	4245	-32719
Subject 6	870	913	-18485
Mean	**-4685**	**-5458**	**-24541**
sem	±2891	±3868	±7787
Confidence interval (95%)	±5173	±6919	±10 001

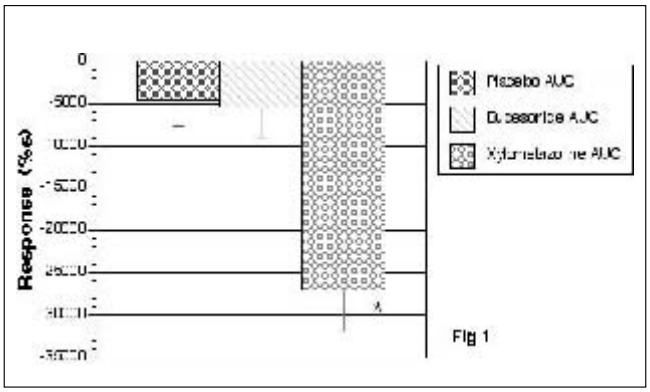

Figure 1. Budesonide does not affect blood flow compared to placebo which is in contrast to xylometazoline. The LDF recordings of challenge with placebo, budesonide 64 μg, and xylometazoline 140 μg, measured as, mean area under curve during 20 minutes (%s, ±sem), n=6. P<0.05 for xylometazoline compared to placebo or budesonide.

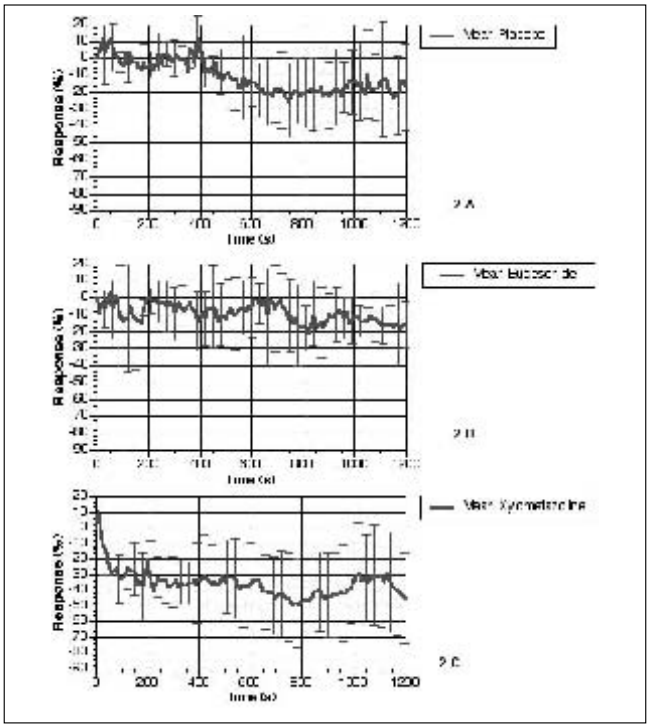

Figure 2 a-c. The time course curves (means±sem, please note that for clarity sem is not displayed for all data points) for placebo spray, n=6 (A), budesonide 64 μg spray, n=6 (B) and the positive control, xylometazoline 140 μg spray, n=6 (C). P<0.05 for xylometazoline compared to placebo or budesonide. Note the small increase in blood flow during the first 60 sec in all recordings.

by micrometer screws and a ball-and -socket-joint, with an accuracy of approximately 0.5 mm. The probe was advanced into the nose under direct supervision with a 30° rigid endoscope. Local anesthesia was not used. The tip of the probe was directed towards the septal mucosa at a level corresponding to the anterior edge of the middle turbinate and positioned approximately 1 mm from the mucosa, and never touching the mucosa. The Periflux gain setting was x 1, and the frequency limit set at 12 KHz. The LDF signal was continuously recorded by a penwrite recorder and by computer every 10 s.
The patients were first acclimatized to room temperature for at least 15 minutes, then after a stable 3-5 min. baseline level had been established, the spray dose was given. The recordings ended 20 minutes after dosing.

Drugs and administration
The volunteers were randomly allocated to a unilateral single dose of budesonide aqueous nasal spray 64 μg per puff (50 μl) (Rhinocort® Aqua, AstraZeneca R&D Lund, Sweden) and placebo, identical in appearance and taste. A unilateral single dose of xylometazoline-hydrochloride (Nezeril® 1.0 mg/ml, AstraZeneca, Lund, Sweden) was used as positive control. The nasal sprays were given without removing the LDF probe.

Experimental procedure
The subjects were seated in position for LDF measurements with the chin and forehead in a head rest. The LDF probe was positioned inside the nose and blood flow was registered until the signal was stable for 3 minutes. The spray pumps were primed with three puffs, and then one puff of budesonide or placebo was administered into the nasal cavity without touching the LDF probe. The blood flow was measured continuously for at least another 20 minutes. Each session was separated from the next by a minimum of 3 days. The procedure was identical at all three sessions.

Statistics
Blood flow changes were expressed as percentages of the mean of the baseline LDF signal during the 60 sec preceding challenge. Results are expressed as means ± standard error of the mean. Comparison between groups were made with the three way ANOVA test.

RESULTS
The term blood flow is used throughout to describe the signal registered by the LDF probe from the nasal septal mucosa. The basal blood flow for placebo was 44.4±4.7 AU (arbitrary units); for budesonide: 47.0±2.9 AU; for xylometazoline 40.2±3.3 AU. There were no differences in basal blood flow between any of the sessions. During the first 60 sec after the sprays had been delivered a small, but non-significant increase in blood flow was seen after both placebo, budesonide, and xylometazoline. During the 20 minutes recording after the administration of the sprays no significant differences between budesonide and placebo were found (Figures 1, 2a, 2b and Table 1). Xylometazoline induced a clear reduction of 60.9±7.1% ($p<0.05$ as compared to placebo). The reduction appeared within 1 minute and was fully developed at approximately 3 minutes (Figure 2 c and Table 1). The reduction was sustained throughout the whole registration.

DISCUSSION
Budesonide did not cause acute reduction of the nasal septal blood flow as measured with LDF. There was an initial increase in blood flow which has been registered in the same experimental set-up during nasal challenges with the potent vasoconstrictor Neuropeptid Y and is probably related to the mechanical impact of the spray-droplets on the nasal mucosa causing vasodilation (Cervin et al., 1999). It has also been shown that mechanical tactile stimulation, by the probe itself increases the perfusion (i.e. blood flow) (Grudemo et al., 1997). Over the registration period there was a slight decrease in blood flow after budesonide and placebo alike. This has been observed previously and could be due to fact that the subject gradually relaxes during the registration period. We have previously observed subjects falling asleep during a blood flow registration, coinciding with a decline in blood flow, which has been restored when the subject was alerted.

The nasal mucosal vascular bed can functionally be divided into two types: resistance vessels and capacitance vessels. A constriction of resistance vessels lead to a reduction in the nutritive flow while a capacitance vessel constriction will lead to decongestion. The Laser Doppler method is sensitive to small and rapid changes in blood flow foremost regulated by the resistance vessels. LDF is totally non-invasive and the probe to mucosa distance may vary up to 3.5 mm without affecting the signal (Olsson, 1986).

When glucocorticoids are applied to the skin a blanching phenomenon occurs. This has been interpreted as a vasoconstriction and has been widely used in the grading of glucocorticoid potency (McKenzie, 1962). It was therefore conceivable to anticipate a similar phenomenon in the nasal mucosa. Even if we were unable to demonstrate any vascular effects caused by GCS such as a decreased nasal blood flow, such a phenomena cannot be excluded in the nasal mucosa. The glucocorticosteroid effect may have a slow onset and thus not being possible to detect within 20 minutes. Application of higher glucocorticosteroid concentrations than those reached by the clinical doses in the nasal mucosa may be necessary for vasoconstriction. The retention time of budesonide in the nasal mucosa could be shorter than in the skin (Harris, 1975). The basal blood flow in the nasal mucosa, with a rich submucosal capillary network, is much higher than in the skin. Hence, budesonide could be faster removed from the local tissue. A recent hypothesis put forward in the field of dermatology is that corticosteroids in the skin mainly affects the venules thus explaining the lack of correlation seen between the skin blanching test and LDF (Andersen et al., 1993). However, an effect on venules would cause a reduction in nasal airway resistance which was not the case in a previous study (Lindqvist et al., 1989). A recent study using

inhaled fluticasone propionate revealed a decrease in deadspace after 30 minutes suggesting a vasoconstrictive effect which may have affected the venules causing decongestion of the mucosa. This is further supported by the fact that the effect was more pronounced in asthma subjects than in healthy volunteers (Kumar et al., 2000).

The only method which has previously been used to assess effects on resistance vessels in the nasal mucosa after a nasal steroid challenge is the ^{133}Xenon wash-out technique (Bende et al., 1983). It involves injecting a radioactive isotope in the mucosa and it is not possible to detect small and rapid changes in blood flow with this method. In the study by Bende et al., (1983) the blood flow was measured 2 hours after administration of budesonide and did not show any changes. It is also believed that the ^{133}Xenon wash-out method reflects the deeper vessels whereas LDF measures the more superficial vessels (Olsson, 1986).

The effect of xylometazoline in our study is in accordance with previous LDF studies in both animal and man. In the rabbit maxillary sinus a decrease between 50 and 70% was noted (Akerlund et al., 1993; Cervin et al., 1988), and in the human nasal mucosa, a 60% reduction of blood flow by 100 μg of xylometazoline was seen (Olsson, 1986). The results indicate the sensitivity and consistency of LDF measurements. The rapid and pronounced decrease in blood flow by a positive control validate the experimental set-up and the conclusion that budesonide is unlikely to impair nutritive blood flow since no blood flow changes were detectable. Based on the present study it cannot be ruled out that the long-term effect of intranasal corticosteroids may affect blood flow in the nasal mucosa. One has also to bear in mind that this study was performed on healthy volunteers and that the vessels of patients with inflamed mucosa may react differently. However, to assess changes over longer time periods, another method than LDF has to be used. Registration periods exceeding 30 minutes are not possible due to discomfort for the study subject. Hence, in the present study we used the longest possible registration time, not causing any discomfort for the subjects.

In conclusion, a single dose of intranasal budesonide had no acute effects on nasal septal mucosal blood flow. Neither onset of action within a few hours, nor any potentially unfavourable effects are likely to be caused by vasoconstriction.

ACKNOWLEDGEMENT

The assistance of laboratory technician Charlotte Cervin-Hoberg is greatly appreciated. The study was supported in parts by grants from the Swedish Medical Research Council (project no: 12907, 07940, and 8308), Vårdal Foundation, and the Swedish Society of Medicine.

REFERENCES

1. Akerlund A, Arfors KE, Bende M, Intaglietta M (1993) Effect of oxymetazoline on nasal and sinus mucosal blood flow in the rabbit as measured with laser-Doppler flowmetry. Ann Otol Rhinol Laryngol 102(2):123-126.
2. Andersen P, Milioni K, Maibach H (1993) The cutaneous corticosteroid vasoconstriction assay: a reflectance spectroscopic and laser-Doppler flowmetric study. Br J Dermatol 128(6):660-665.
3. Bende M, Lindqvist N, Pipkorn U (1983) Effect of a topical glucocorticoid, Budesonide on nasal mucosal blood flow as measured with 133Xe wash-out technique. Allergy 38:461-446.
4. Cervin A, Andersson M (1998) Intranasal steroids and septumperforation - an overlooked complication? A description of the course of events and a discussion of the causes. Rhinology 36:128-132.
5. Cervin A, Bende M, Lindberg S, Mercke U, Olsson P (1988) Relations between blood flow and mucociliary activity in the rabbit maxillary sinus. Acta Otolaryngol (Stockh) 105:350-356.
6. Cervin A, Onnerfalt J, Edvinsson L, Grundemar L (1999) Functional effects of neuropeptide Y receptors on blood flow and nitric oxide levels in the human nose. Am J Respir Crit Care Med 160(5 Pt 1):1724-1728.
7. Day JH, Briscoe MP, Rafeiro E, Ellis AK, Pettersson E, Akerlund A (2000) Onset of action of intranasal budesonide (Rhinocort aqua) in seasonal allergic rhinitis studied in a controlled exposure model. J Allergy Clin Immunol 105(3):489-494.
8. Grudemo H, Juto JE (1997) The impact of the measuring distance on Laser-Doppler measurements of the microcirculation in human nasal mucosa. A study of rhinostereometry and micromanipulator-guided Laser-Doppler flowmetry. ORL J Otorhinolaryngol Relat Spec 59(5):280-285.
9. Harris DM (1975) Some properties of beclomethasone dipropionate and related steroids in man. Postgrad. Med. J. Suppl. 4:20-25.
10. Kumar SD, Brieva JL, Danta I, Wanner A (2000) Transient effect of inhaled fluticasone on airway mucosal blood flow in subjects with and without asthma. Am J Respir Crit Care Med 161(3 Pt 1):918-921.
11. Linden M, Svensson C, Andersson E, Andersson M, Greiff L, Persson CG (2000) Immediate effect of topical budesonide on allergen challengeinduced nasal mucosal fluid levels of granulocyte-macrophage colony-stimulating factor and interleukin-5. Am J Respir Crit Care Med 162:1705-1788.
12. Lindqvist N, Holmberg K, Pipkorn U (1989) Intranasally administered budesonide, a glucocorticoid, does not exert its clinical effect through vasoconstriction. Clin Otolaryngol 14(6):519-523.
13. McKenzie AW (1962) Percutaneous absorption of steroids. Arch. Dermatol. 86:611.
14. Olsson P (1986) A comparison between the 133Xe wash-out and Laser Doppler techniques for estimation of nasal mucosal blood flow in humans. Acta Otolaryngol (Stockh) 102:106-112.
15. Olsson P, Bende M, Ohlin P (1985) The laser Doppler flowmeter for measuring microcirculation in human nasal mucosa. Acta Otolaryngol (Stockh) 99:133-139.
16. Sommer A, Veraart J, Neumann M, Kessel A (1998) Evaluation of the vasocontrictive effects of topical steroids by laser-Doppler-perfusion imaging. Acta Derm Venereol 78:15-18.
17. Tenland T (1982) On laser Doppler flowmetry. Methods and microvascular applications. [Medical Dissertation No. 136]: Thesis. Linköping University, Sweden.

Anders Cervin
Dept of Oto-Rhino-Laryngology,
Head and Neck Surgery,
University Hospital of Lund,
S-221 85 Lund,
Sweden
Fax: + 46-46211-0968
E-mail: anders.cervin@onh.lu.se