

Short communication

Nasal nitric oxide, the guardian of paranasal sinuses, is paradoxically increased by high doses of intravenous glucocorticoids

Background: High concentrations of nitric oxide (NO) originating from a type-2 nitric oxide synthase (NOS2) located within the paranasal sinuses are measured in nasal air in man. NO is believed to play a central role in nonspecific defense of paranasal sinuses. Glucocorticoids (GCs), a therapeutic often used for a wide range of diseases, is known to strongly downregulate NOS2.

Aims of the study: To investigate the effect of very high intravenous doses of GCs on nasal NO in man.

Methods: Nasal NO was measured in 15 patients without any history of allergy or chronic airway disorder who were treated for 3 days with a daily dose of 1000 mg methylprednisolone for an exacerbation of multiple sclerosis. Nasal NO was also measured in 30 matched control subjects.

Results: In control subjects, the maximal value of nasal NO [mean (SE)] was 233 (8) part per billion (ppb), and did not differ from patients with multiple sclerosis [maximum value: 219 (13) ppb; left nostril: 214 (12) ppb; right nostril: 215 (12) ppb]. After GCs treatment, nasal NO increased in patients [maximum value: 250 (13) ppb ($P < 0.0001$); left nostril: 249 (12) ppb ($P < 0.0001$); right nostril: 244 (13) ppb ($P < 0.0001$)].

Conclusions: We conclude that GCs do not decrease but even increase nasal NO.

B. Degano^{1,2}, L. Têtu^{1,3}, E. Serrano^{3,4}, A. Didier^{1,3}, J. -F. Arnal²

¹Service de Pneumologie, CHU Larrey; ²INSERM U589, CHU Rangueil; ³Groupe de recherche en physiopathologie de l'inflammation des voies aériennes de la Clinique des Voies Respiratoires, CHU Larrey; ⁴Service d'Otorhinolaryngologie, CHU Larrey, Toulouse, France

Key words: epithelium; nitric oxide.

Bruno Degano
CHU Larrey
TSA 30030, 31059
Toulouse Cedex 4
France

Accepted for publication 20 March 2005

Glucocorticoids (GCs) are commonly given for a wide range of respiratory diseases including nasal and paranasal sinuses diseases (1). Very high doses of GCs are also given to patients with multiple sclerosis who present with an exacerbation (2). GCs are employed as anti-inflammatory and immunosuppressive drugs, because of their multiple inhibitory effects on the immune system. In most cases, GCs also strongly and rapidly inhibit the expression of the type 2 nitric oxide synthase (NOS2) (3). Nitric oxide (NO) generated by NOS2 has a major but complex role in nonspecific host defense in humans. In most organs, basal expression of NOS2 and thereby basal NO concentrations are very low, while in case of inflammation or infection, NOS2 expression is induced and NO acts as a second line of defense (4). The scenario in the paranasal sinuses appears to be the reverse: the epithelium constitutively expresses NOS2, leading to NO concentrations greater than 20,000 part per billion (ppb) (5), whereas inflammation or infection of the sinus almost completely suppress paranasal NO production by down regulation of NOS2 (6).

As GCs are believed to downregulate NOS2 in humans, we aimed to investigate the effect of very high intravenous doses of GCs on the production of NO

within the paranasal sinuses. NOS2 located in the paranasal sinuses is the major source of the NO found in the nasal cavity in mammals (7) and healthy adults (5, 8). Contrarily to the direct measurement of sinus NO, measurement of nasal NO is a noninvasive and repeatable technique. We therefore prospectively measured nasal NO in patients without any respiratory disease who received intravenous GCs for an exacerbation of multiple sclerosis.

Material and methods

The study protocol was approved by the local ethics committee (CCPRB Toulouse 2). We included fifteen consecutive nonsmoking patients admitted for an exacerbation of multiple sclerosis [five men and 10 women, mean (SE) age 34 (2) years], and 30 non-smoking matched control subjects. Control subjects [13 men and 17 women, mean (SE) age 31 (3) years] were members of the medical staff of the Rangueil-Larrey Hospital. Patients and control subjects did not have any history of allergy or chronic airway disorder. They were verified to have bilateral ostium patency with a bilateral endoscopy performed by an otorhinolaryngologist. In patients, nasal NO was measured just before they receive methylprednisolone (1000 mg/day for at least 3 days) prescribed for the exacerbation of

multiple sclerosis, and 72–80 h after the onset of treatment. Ten of 15 patients were treated with interferon-beta-1b (Betaferon®, Schering AG, Berlin, Germany) for more than 2 months. Controls and patients did not take GCs any route for at least 2 months before entering the study. To measure nasal NO, we used a chemiluminescence NO analyzer (Cosma, Igny, France) sampling with a constant flow of 0.7 l/min. The probe was connected to a nasal olive and gently introduced into the vestibulum of one nostril. The contralateral nostril was left open. Patients were asked to breathe through the mouth, without speaking or swallowing. Measurements were performed in both nostrils. The atmospheric NO in the room was controlled to be inferior to 5 ppb. The reproducibility of the measure had been assessed in the control subjects by repeating it after 15 min on the same day and by repeating it every morning from Monday to Friday (day-to-day). The 15-min and day-to-day coefficients of variation were 4 and 6%, respectively (data not shown). Comparisons of data among patients were made by paired student's *t*-test, and comparisons between patients and controls were made by unpaired student's *t*-test. Comparisons of data between patients receiving (*n* = 10) or not receiving (*n* = 5) interferon-beta-1b were made by the nonparametric *U*-test of Mann and Whitney. Statistical significance was accepted for values of *P* < 0.05.

Results

In normal subjects (control group), the maximal value of nasal NO [mean (SE)] was 228 (15) ppb, and did not differ from patients with multiple sclerosis [max value: 219 (13) ppb; left nostril: 214 (12) ppb; right nostril: 215 (12) ppb]. There was no difference in nasal NO between patients receiving or not interferon-beta-1b either before or after GCs (Table 1). After GCs treatment, nasal NO increased in patients [maximum value: 250 (13) ppb, *P* < 0.0001; left nostril: 249 (12) ppb, *P* < 0.0001; right

nostril: 244 (13) ppb, *P* < 0.0001] (Figs 1 and 2). Nasal NO in patients after GCs (maximum value, left nostril and right nostril) did not differ from nasal NO in controls (maximum value).

Discussion

This result is the first demonstration that GCs, given at very high doses for a short time in subjects without upper airway disease, do not decrease but even increase nasal NO. To the best of our knowledge, the effect of GCs on nasal NO was not previously studied prospectively. Lundberg et al. retrospectively compared nasal NO of five patients taking a chronic treatment with oral GCs because of solid brain tumors (betamethasone, 4–16 mg/day) with

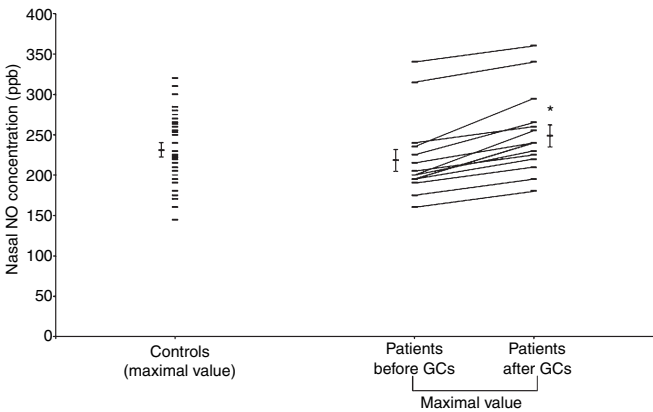


Figure 1. Nasal nitric oxide (NO) concentration [parts per billion (ppb)] in control subjects and in patients. The higher value of nasal NO obtained after measurements in both nostrils was recorded (maximal nasal NO). Maximal nasal NO increased in patients after treatment with glucocorticoids (GCs) (**P* < 0.0001 vs patients before GCs).

Table 1. Nasal NO in patients treated (*n* = 10) or not (*n* = 5) with interferon-beta-1b, before and after treatment with glucocorticoids (GCs)*

		Nasal NO (ppb)			
		Before GCs		After GCs	
	Treatment with interferon-beta-1b	Left nostril	Right nostril	Left nostril	Right nostril
Patient 1	No	200	180	230	205
Patient 2	Yes	340	320	355	360
Patient 3	Yes	160	160	180	180
Patient 4	Yes	165	175	195	195
Patient 5	Yes	200	215	240	235
Patient 6	Yes	190	190	210	210
Patient 7	No	220	235	295	290
Patient 8	Yes	195	190	240	230
Patient 9	Yes	205	205	225	225
Patient 10	Yes	190	195	235	240
Patient 11	No	300	315	330	340
Patient 12	Yes	225	220	265	240
Patient 13	Yes	185	195	220	210
Patient 14	No	200	200	255	255
Patient 15	No	240	230	260	250

*There was no significant difference in nasal NO at any time according to the treatment with interferon-beta-1b (*U* test of Mann–Whitney).

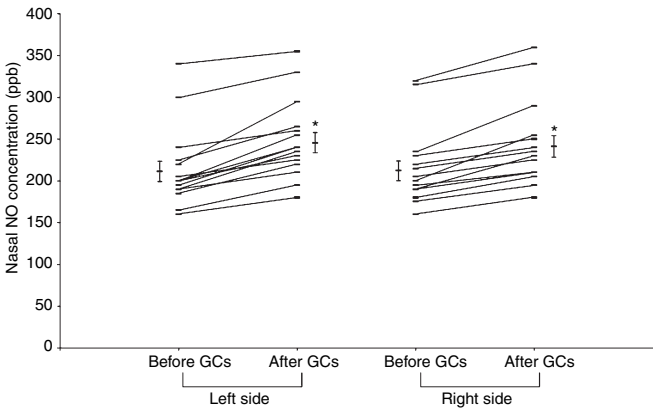


Figure 2. The concentration of nasal NO [parts per billion (ppb)] increased in both nostrils in patients after treatment with GCs (**P* < 0.0001 vs patients before GCs).

nasal NO of nine controls (9). The authors concluded that GCs had no influence on nasal NO, although the patient number was very small and the study had retrospective design. In accordance with Lundberg et al., we did not find any significant difference in nasal NO between controls and patients after GCs. Only a paired comparison among patients showed an effect of GCs on nasal NO.

Glucocorticoids are a class of steroid hormones with pleiotropic effects. At pharmacological concentrations, GCs are used to prevent and suppress inflammation (10). Glucocorticoids exert their anti-inflammatory actions mainly by modulation of the transcription of a variety of genes involved in the control of inflammatory processes (10). In phagocytes, GCs inhibit the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that produces superoxide anion. Phagocyte-derived superoxide anion can interact with NO and then contribute to decrease nasal NO (11). GCs may at least partly decrease the interaction of NO with superoxide anion (and in that way increase NO availability) via the inhibition of NADPH-oxidase. This mechanism must, however, be of little importance in subjects without nasal disease where phagocytic cells are rare in the nasal cavity.

It can be speculated that the rise in nasal NO after GCs could be due, at least in part, to an improvement of the communication between paranasal sinuses and nasal cavity. Indeed, it was clearly shown that in patients with ostia occluded by polyps, improvement of the communication between the sinuses and the nasal cavities after polypectomy may increase nasal NO (12, 13). Moreover, we only verified ostium patency with a nasendoscopy, because the experimental design of our study did not allow us to perform a precise assessment of ostium by CT or MRI scan. However, the contribution of an increase of ostium patency to the rise in nasal NO is not certain for at least to reasons. First, patients and controls were free from any history of allergic or obstructive upper airway disease. Second, Weitzberg et al. suggested that nasal NO measured during quiet breathing in subjects with patent ostia does not depend on the size of the ostia (14).

We studied 15 patients with multiple sclerosis, among whom 10 were treated with interferon-beta. We did not

find any influence of this latter treatment on nasal NO at any time. Interferon-beta has however been reported to interfere with the production of NO and the expression of NOS2, but the findings depend on the model. Interferon-beta acts as a co-activator of NOS2 expression in murine macrophages (15), but decreases the production of NO in human astrocytoma cells (16) and the expression of NOS2 in human glial cells (17).

It was largely demonstrated that GCs decrease NOS2 transcription in inflammatory conditions (18). However, patients with maxillary sinusitis and sepsis who had considerably lower maxillary NO concentrations than controls before sinus fenestration increased their maxillary sinus NO release in the time period following fenestration despite of ongoing corticosteroid therapy (6). In particular, the single patient who reached a maxillary sinus NO concentration in the range of the controls after fenestration still received hydrocortisone at a dose of 250 mg/day at the time of the measurement (6). Last but not least, it was demonstrated that dexamethasone enhanced NO production by the human astrocytoma cell line (16). Then, the hypothesis that GCs may increase paranasal and/or nasal NO production in our study population cannot be discarded.

Glucocorticoids are among of the most often used therapies for sinus and nasal diseases, especially in chronic inflammation. It is possible that the spectacular efficacy of GCs is due, at least in part, to the preservation of the first line of host defense (i.e. high NO) that appears to be specific to this anatomic site. However, despite short-term beneficial effects, long-term effects of GCs should be considered due to the numerous effects on gene expression.

Acknowledgments

We are especially indebted to Doctor Mathieu Rigal, from the Department of Neurology of Rangueil, and to Doctor Frédérique Viala, from the Department of Neurology of Purpan, who kindly helped us to recruit the patients.

This work was sponsored by the University Hospital of Toulouse for regulatory and ethic submissions.

References

1. Dykewicz MS. 7. Rhinitis and sinusitis. *J Allergy Clin Immunol* 2003;**111**:S520–S529.
2. Keegan BM, Noseworthy JH. Multiple sclerosis. *Annu Rev Med* 2002;**53**:285–302.
3. Kunz D, Walker G, Eberhardt W, Pfeilschifter J. Molecular mechanisms of dexamethasone inhibition of nitric oxide synthase expression in interleukin 1 beta-stimulated mesangial cells: evidence for the involvement of transcriptional and posttranscriptional regulation. *Proc Natl Acad Sci U S A* 1996;**93**:255–259.
4. Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *Proc Natl Acad Sci U S A* 2000;**97**:8841–8848.
5. Lundberg JO, Farkas-Szallasi T, Weitzberg E et al. High nitric oxide production in human paranasal sinuses. *Nat Med* 1995;**1**:370–373.

6. Deja M, Busch T, Bachmann S et al. Reduced nitric oxide in sinus epithelium of patients with radiologic maxillary sinusitis and sepsis. *Am J Respir Crit Care Med* 2003;**168**:281–286.
7. Lewandowski K, Busch T, Lohbrunner H et al. Low nitric oxide concentrations in exhaled gas and nasal airways of mammals without paranasal sinuses. *J Appl Physiol* 1998;**85**:405–410.
8. Arnal JF, Flores P, Rami J et al. Nasal nitric oxide concentration in paranasal sinus inflammatory diseases. *Eur Respir J* 1999;**13**:307–312.
9. Lundberg JO, Weitzberg E, Rinder J et al. Calcium-independent and steroid-resistant nitric oxide synthase activity in human paranasal sinus mucosa. *Eur Respir J* 1996;**9**:1344–1347.
10. Pujols L, Mullol J, Torrego A, Picado C. Glucocorticoid receptors in human airways. *Allergy* 2004;**59**:1042–1052.
11. Pasto M, Serrano E, Urocoste E et al. Nasal polyp-derived superoxide anion: dose-dependent inhibition by nitric oxide and pathophysiological implications. *Am J Respir Crit Care Med* 2001;**163**:145–151.
12. Ragab SM, Lund VJ, Scadding G. Evaluation of the medical and surgical treatment of chronic rhinosinusitis: a prospective, randomised, controlled trial. *Laryngoscope* 2004;**114**:923–930.
13. Colantonio D, Brouillette L, Parikh A, Scadding GK. Paradoxical low nasal nitric oxide in nasal polyposis. *Clin Exp Allergy* 2002;**32**:698–701.
14. Weitzberg E, Lundberg JO. Humming greatly increases nasal nitric oxide. *Am J Respir Crit Care Med* 2002;**166**:144–145.
15. Jacobs AT, Ignarro LJ. Lipopolysaccharide-induced expression of interferon-beta mediates the timing of inducible nitric-oxide synthase induction in RAW 264.7 macrophages. *J Biol Chem* 2001;**276**:47950–47957.
16. Guthikonda P, Baker J, Mattson DH. Interferon-beta-1-b (IFN-B) decreases induced nitric oxide (NO) production by a human astrocytoma cell line. *J Neuroimmunol* 1998;**82**:133–139.
17. Hua LL, Liu JS, Brosnan CF, Lee SC. Selective inhibition of human glial inducible nitric oxide synthase by interferon-beta: implications for multiple sclerosis. *Ann Neurol* 1998;**43**:384–387.
18. Radomski MW, Palmer RM, Moncada S. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci U S A* 1990;**87**:10043–10047.