Serum nitric oxide products in different allergic diseases

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Abstract: We assessed serum nitric oxide products, nitrites and nitrates in 61 controls and 39 patients with atopic diseases: 14 with allergic rhinitis (RHIN), 19 with urticaria (URT) and six with rhinitis plus bronchial asthma (RHIN+ASTH). The patients with URT had the lowest levels of NO products as compared to controls ($P < 0.0001$) and to other groups ($P = 0.0001$). Patients with RHIN had lower levels of nitrates and total NO products as compared to controls ($P < 0.01$) and significantly lower levels of nitrites as compared to RHIN+ASTH patients patients. On the contrary, the levels of NO products were similar in the RHIN+ASTH groups as compared to the controls despite a significantly higher level of nitrites ($P < 0.05$) and lower level of nitrates ($P = 0.05$). There was no direct correlation between NO levels with either IgE values or sex or age. Serum NO levels may vary depending on the atopic disease studied.

Keywords: nitric oxide, atopy, allergic rhinitis, urticaria, bronchial asthma, nitrate, allergy

Introduction: Different leucocyte subpopulations, along with numerous endogenous substances produced by them, have been studied in allergic diseases. However, the role of certain widespread inflammatory products has been only partially addressed. Nitric oxide (NO) is one of these metabolites.

NO is produced by the enzymatic conversion of arginine to citrulline using NADPH [1,2]. Three nitric oxide synthases (NOS) have been described up to date: one endothelial, one neuronal and the other inducible (related to immune response) [1,2]. Previous reports [3–11] have shown an increment in the exhaled air of patients with asthma, rhinitis or bronchiectasis, and individuals with upper respiratory tract infection. However, the systemic production of NO has not been monitored in allergic diseases.

Due to the widespread function of NO, the aim of the study was to assess the importance of this metabolite in distinct allergic diseases.

Materials and methods: Blood samples from 39 patients (mean age 33 ± 8 years), from the outpatient clinic of the Institute of Immunology, Caracas, and 61 controls (34 ± 9 years) were obtained after securing each patient's written consent and the approval of the Local Ethical Committee. The controls did not suffer from any chronic, viral, parasitic or genetic disease.

The patients were classified as: allergic rhinitis (RHIN), urticaria (URT) and allergic RHIN plus bronchial asthma (ASTH) according to clinical, physical and paraclinical parameters of allergy (nasal cytology, serum IgE levels and eosinophil count per mm^3). We excluded patients with autoimmune diseases, diabetes or another chronic, viral or systemic disease, those with positive antinuclear-antibodies, and those older than 60 years or younger than 16 years.

Most of the patients with URT (80%) received antihistaminic treatments. The patients in the other groups were not medicated at the time of sample collection. The blood samples were taken during fasting, for no less than 4 h and no longer than 24 h. The patients' food intake was similar. All patients and controls received a diet with low contents of nitrate and nitrite following the guidelines described elsewhere [12].

Nitric oxide metabolites (nitrite and nitrate) in serum were assessed according to the method of Moshage *et al.* [13]. Total products were quantified after nitrate reductase (from *Aspergilus* spp) incubation and total nitrite was determined using the Greiss reagent [14]. Nitrate values were obtained by subtracting basal nitrite values from total products.

IgE levels were measured by radioimmune assay (RIA) using a commercial kit (Kallestat diagnostics,Sanofi Pasteur Diagnostics, Chaska, Minnesota, USA).

The different groups were compared using ANOVA and Student's *t*-test. Linear regression analysis was used to determine a possible correlation between nitrites/nitrates and IgE or age. Chi squared was used to compare sex frequency between groups.

Results: Table 1 illustrates the characteristics of patients and controls. There were no differences in sex or age distribution between groups ($P < 0.2$). The levels of IgE were significantly higher in the different groups of patients as compared to controls ($P < 0.05$ for RHIN, $P < 0.0001$ for URT and $P < 0.0001$ for RHIN+ASTH). The levels of IgE in the RHIN+ASTH group were also significantly higher ($P < 0.05$) as compared to patients in the RHIN group.

Table 1 shows levels of nitrites, nitrates and the sum of both metabolites for all groups. There was no correlation between IgE levels and nitrites or nitrates or the sum of these metabolites in any group ($r < 0.2$ for any condition). Neither was there any correlation between sex or age with nitrites or nitrates levels ($r < 0.2$ for any condition).

Nitrite levels were significantly lower in the URT group ($P < 0.0001$) and significantly higher in rhinitis plus asthma (RHIN+ASTH) group as compared to controls ($P < 0.5$). Nitrate levels were lower in the RHIN group ($P < 0.005$), the URT group ($P < 0.0001$) and in the RHIN+ASTH group

Table 1. General characteristics of the patients studied (mean ± SEM)

	Controls	RHIN	URT	RHIN+ASTH
n	61	14	19	6
Age (years)	34 ± 9	33 ± 9	38 ± 10	32 ± 16
% Female	62	57	79	67
IgE (U/dl)	110 ± 20	277 ± 358 *	377 ± 393 ***	557 ± 496 ***
Nitrites (μmol/l)	7.1 ± 2.0	6.2 ± 1.6	4.6 ± 1.4	9.0 ± 3.9
Nitrates (μmol/l)	18.9 ± 2.8	16.0 ± 4.8	12.7 ± 3.0	16.2 ± 1.6
Nitrites + Nitrates (μmol/l)	26.0 ± 4.6	22.2 ± 5.0	15.2 ± 4.1	25.2 ± 4.3

Rhinitis (RHIN); urticaria (URT); rhinitis plus asthma (RHIN+ASTH).
As compared to the controls *P < *0.05*, ***P < *0.0001*.

($P < 0.05$) in comparison to controls. On the contrary, when the sums of the metabolites were compared, only the groups of patients with RHIN ($P < 0.01$) and URT ($P < 0.0001$) were significantly lower than the controls.

ANOVA analysis, represented in Table 2, revealed significant differences in any NO metabolites ($P < 0.0001$). However, the statistical differences depended on the metabolite analysed and the group compared. As an example, there was no significant difference when the values of nitrates were compared between the control and RHIN group ($P = 0.13$). However, the difference was significant in nitrates ($P = 0.003$) and in the sum of metabolites ($P = 0.006$). The contrary (significance in nitrite and nitrate levels but no difference in the sum of metabolites) was observed in the RHIN+ASTH group as compared to the control.

Discussion: NO plays an important role in cell signalling and has been implicated in the pathophysiology of several diseases [1,2]. In inflammation, an event related to the severity of asthma, rhinitis and others allergic diseases, the transcription of iNOS is upregulated by inflammatory cytokines [3–11]. This leads to an increase in NO production, which may be responsible for the severity of the lesion [3–11].

There have been few studies on the role of NO in allergic diseases [3–11]. In asthmatic patients, NO was quantified from exhaled air and found to be increased as compared to normal controls [3–5]. Furukawa *et al.* [6] demonstrated that iNOS is expressed in airway epithelial cells from asthmatic patients but it is absent in normal individuals. In concordance, Garrels *et al.* [7] showed a progressive increment in nitrites and nitrates, after provocation with house dust mite extract, in nasal lavages from patients with allergic RHIN to house dust mite.

Table 2. ANOVA analysis of the different groups
P for nitrites, nitrates and nitrites + nitrates < 0.0001

vs		RHIN	URT	RHIN+ASTH
Control	Nitrites	0.13	< 0.0001	0.04
	Nitrates	0.003	< 0.0001	0.05
	Total	0.006	< 0.0001	0.7
RHIN	Nitrites		0.03	0.01
	Nitrates		0.005	> 0.8
	Total		0.0001	0.2
URT	Nitrites			0.0001
	Nitrates			0.03
	Total			0.0001

The table represents the significance of P *(two-tailed) observed in the comparison between the different groups. The first line of each comparison represents nitrites, the second line nitrates and the third line the sum of total NO products*

Martin *et al.* [8] observed significantly higher exhaled NO levels in patients with seasonal RHIN. Kharitonov *et al.* [9] showed that nasal glucocorticoids, inhibitors of iNOS transcription, modulated increased NO produced in patients with asthma and allergic rhinitis. To our knowledge, there have been no previous studies on the role of NO in chronic URT.

In our study, we demonstrated that local and systemic inflammatory diseases altered serum concentrations of NO oxidative products. By measuring the production of nitrites and nitrates, we could differentiate NO metabolism in the different allergic diseases studied. Patients with urticaria had the lowest levels of NO metabolites as compared with the different groups.

In addition, patients with RHIN had significantly lower levels of nitrate and total products as compared to controls, which contrasted to the values observed in the RHIN+ASTH group in which the nitrite levels were higher than in the controls. These results suggest that NO metabolite concentration is not only related to the extent of the inflammatory response, but also to the kinetic of nitrate production and the severity and type of disease.

Three hypothesis may be proposed to explain the low levels of nitrites and nitrates reported in the URT group. These are the different aetiology of URT as compared to the other allergic diseases studied, the possibility that the use of systemic anti-histamine treatments was more frequent in this group when the sample blood was collected, and thirdly the possibility that NO production or metabolism is different in this pathology. The last hypothesis is supported by the fact that inflammatory cytokines down-regulate endothelial NOS (eNOS) [15] and consequently the lower serum levels of NO metabolites in URT may be due to diminished eNOS activity.

Despite a significant difference in IgE levels between the groups, we could not find a direct correlation between NO and IgE levels. IgE values were very variable in the allergic diseases studied in this report. In addition, there was no correlation with other parameters such as age or sex.

We conclude that serum nitrate and nitrite levels may be useful parameters to assess grade and prognosis in many inflammatory diseases.

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