Increased Expression and Activity of MMP-9 in Chronic Rhinosinusitis With Nasal Polyposis

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Introduction: Matrix metalloproteinases (MMP) are a family of endopeptidases involved in extracellular matrix degradation and which could potentially explain specific histological changes in nasal polyposis. The aim of this study is to determine whether MMP-2 and 9 are involved in chronic rhinosinusitis with polyposis.
Material and method: Specimens were collected from 15 patients affected by nasal polyposis and 15 control patients (with turbinoplasty performed). Specimens were processed for determination of protein expression levels by Western-blot and for determination of their enzymatic activity by zymography for both MMPs.
Results: Results showed a higher expression and activity of MMP-9 but not of MMP-2 in specimens from patients with polyposis when compared with controls.
Conclusions: These results support the involvement of MMP-9 in the tissue remodelling characteristic of this disease.

Key words: Nasal polyposis. MMP-9. MMP-2.

Aumento de expresión y actividad de MMP-9 en rinosinusitis crónica con poliposis nasal
Introducción: Las metaloproteinasas de matriz (MMP) son un conjunto de endopeptidasas implicadas en la degradación de la matriz extracelular, que podrían explicar los cambios histológicos característicos de la poliposis nasal. El objetivo de este estudio consiste en determinar la implicación de MMP-2 y MMP-9 en la rinosinusitis crónica con poliposis nasal.
Material y método: Para ello, se tomaron muestras de 15 pacientes afectados de poliposis nasal y muestras de 15 controles intervenidos de turbinoplastia. Las muestras se procesaron para análisis de la expresión de ambas MMP mediante Western blot, y para determinación de su actividad enzimática mediante cimografía.
Resultados: Los resultados mostraron un incremento de la actividad y de la expresión de MMP-9, pero no de MMP-2, en las muestras procedentes de pacientes afectados de poliposis nasal en comparación con los controles.
Conclusiones: Estos resultados apoyarían la implicación de la MMP-9 en la remodelación tisular característica de esta enfermedad.

Palabras clave: Poliposis nasal. MMP-9. MMP-2.

INTRODUCTION

Chronic rhinosinusitis with nasal polyposis (CRSwNP) remains a mystery in terms of its aetiological and physiopathological mechanisms.[1] Its anatomopathological substrate comprises the infiltration of the nasosinusal mucosa by inflammatory cells, particularly eosinophils, and histopathological changes such as thinning of the base membrane, epithelial erosion, and de-structuring of the extracellular matrix.[2] These changes, included within the concept of tissue remodelling shared with asthma,[3] still lack any satisfactory explanation. A recent discovery has been the major role of a family of enzymes called matrix metalloproteinases (MMP) that might partially explain the structural alterations caused by nasal polyps.[4-6] These endopeptidases are capable of degrading the components in the extracellular tissue, as well as playing an acknowledged part as inflammation mediators. This group of endopeptidases, which make up the most important proteolytic system involved in the remodelling of the extracellular matrix,[7,8] is mainly regulated by a set of molecules called tissue inhibitors of metalloproteinases (TIMP) which, by binding with MMP, form inactive dimers.[6]

The involvement of these molecules in CRSwNP is far from being understood. Although the increased expression

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of the MMP-9 isoform has been described in CRNP,[5,9] the role of MMP-2 is still under debate.[10,11] Our goal in this paper is to confirm the increase in the expression of MMP-9 in CRSwNP, as well as to study the magnitude of the activity of this isoform in samples of nasal polyps. Similarly, the presence and activity of MMP-2 in CRSwNP will be verified.

MATERIAL AND METHOD

The samples were obtained from 15 patients diagnosed as having CRSwNP according to the criteria contained in the EPOS3[12] consensus document. Due to poor control of their symptoms, surgical treatment was indicated in all of them between 2006 and 2007. Patients with a diagnosis of cystic fibrosis, asthma, or ASA triad were excluded.

The control samples were taken from 15 patients subjected to turbinoplasty in the same period.

Participants with allergic rhinitis were excluded for this study. Thus, all patients were free from typical allergy symptoms, verified by means of a negative prick test. In addition, none of them reported a prior history of nasosinusal surgery.

None of the participants received treatment for their nasal problem in the 46 weeks prior to the procedure (ie, the moment when they were recruited and included on the waiting list for surgery).

This study was submitted to the hospital's review board and approved, and all of the participants understood and signed the informed consent document approved by the review board.

The samples were obtained in the operating theatre, following orotracheal intubation, prior to the start of the surgical procedure, and immediately stored from fresh at –80°C until they were processed.

After all the samples had been collected, they were homogenized by mechanical dissociation and subsequent sonication.

The expression of the MMP-9 and MMP-2 protein concentrations was analyzed using Western blot. The samples (20 µg of protein) from all patients were separated by means of electrophoresis in a 7% polyacrylamide gel and later transferred to a PVDF membrane. Following incubation of the membrane with specific antibodies for both proteins (rabbit anti-MMP-9 IgG and mouse anti-MMP-2 IgG; Chemicon International), the strips were developed using a commercial kit from ECL (Amersham), following the manufacturer's instructions.

The enzymatic activity of MMP-9 and MMP-2 was determined by zymography in samples from all patients using a protocol described previously.[13]

After densitometry, the results were expressed as optical density (expressed in arbitrary units; AU) of the Western blot expression strips and zymographic digestion. For the statistical analysis, a non-normal distribution was considered for the response variable and so the median of the inter-quartile range (IR) was used as the measure of centralization and dispersion. The median test was used to verify the null hypothesis. The level of statistical significance was set at $P<.05$.

RESULTS

The mean (standard deviation) for the age in the CRSwNP group was 39.6 (11.1) years (range, 26-68), versus 31.8 (7.8) years (range, 19-42) in the control group. The distribution by gender was: 60% males and 40% females in the polyposis group and 53.3% males and 46.7% females in the control group. Of the patients with chronic rhinosinusitis, 20% declared themselves to be smokers (10 or fewer cigarettes/day) versus 33.5% in the control group (only 1 smoked more than half a pack a day). No statistically significant difference was observed in any of the demographic variables of both groups.

With respect to the group of patients with polyposis, 60% had grade III polyposis according to the classification of Lildholt,[14] the others presented grade II.

Analysis of the Expression and Activity of MMP-9

The analysis of the protein concentrations using Western blot showed a greater expression of MMP-9 in the patients with nasal polyposis (median value, 189.3 AU; IR, 162-204.2) with respect to the control group (140.6 AU; IR, 110-144.3) (Figure 1).

This greater expression correlated with greater MMP-9 enzyme activity as determined using zymography; patients affected by CRSwNP showed greater activity (118.3 AU; IR, 101.5-124.2) than those in the control group (85.6 AU; IR, 85-86.5) (Figure 1).

Therefore, in summary, our results showed higher levels of expression and activity of MMP-9 with respect to the control group.

Analysis of the Expression and Activity of MMP-2

The measure of MMP-2 protein expression using Western blot did not reveal statistical significant differences between the group affected by CRSwNP (152.3 AU; IR, 118.7-185.9) and the control group (136 AU; IR, 104-149.7) (Figure 2).

In the same way, no differences were found either in the assessment the enzymatic activity using zymography. The optical density obtained was 85.1 AU (IR, 83.3-89) in the patients with CRSwNP versus 85 AU (IR, 83.4-87) in the control group (Figure 2).

DISCUSSION

Recent articles have described the increase in the expression of several isoforms of a group of enzymes called matrix metalloproteinases in CRSwNP, including, MMP-2[11] and MMP-9.[9] A greater expression of the latter has also been associated, in patients who underwent endoscopic nasosinusal surgery, with poor evolution of the healing[15] (greater expression was associated with a higher number of sores and oedema after surgery). Nonetheless, on the other hand, a higher value was found for the MMP-9/TIMP-1 quotient in the patients with a better clinical course.[2] With respect to MMP-2, while some authors have found higher degrees of expression in patients affected by CRNP,[11] others have not detected such a difference.[2]

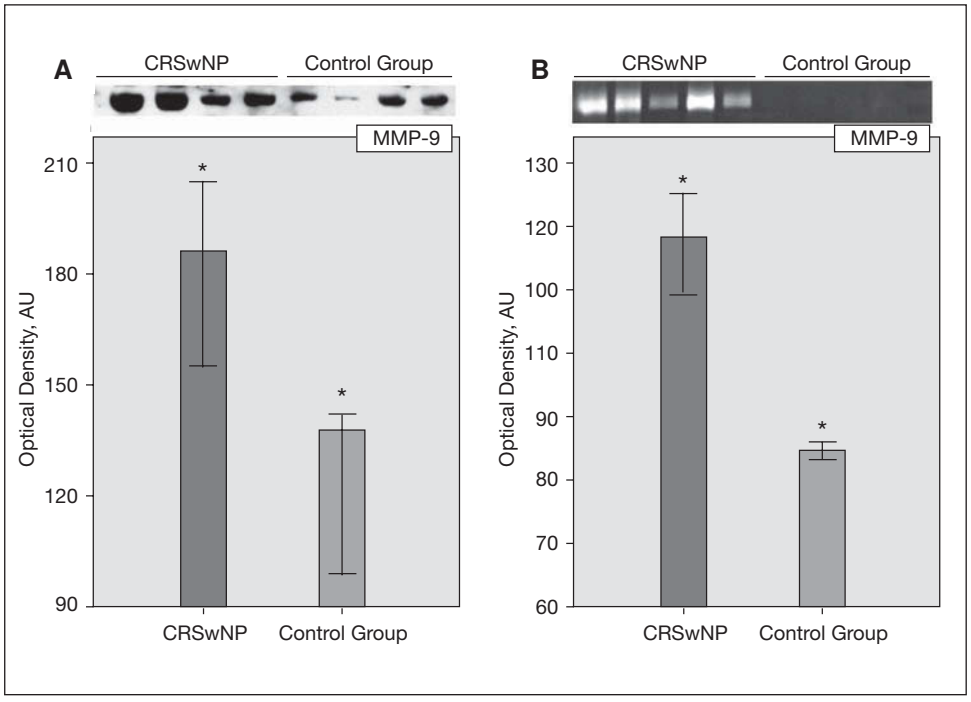

Figure 1. Western blot (A) and zymography (B) results for MMP-9. AU indicates arbitrary units; CRSwNP, chronic rhinosinusitis with nasal polyposis. *Null hypothesis verification: median test, *P*<.05.

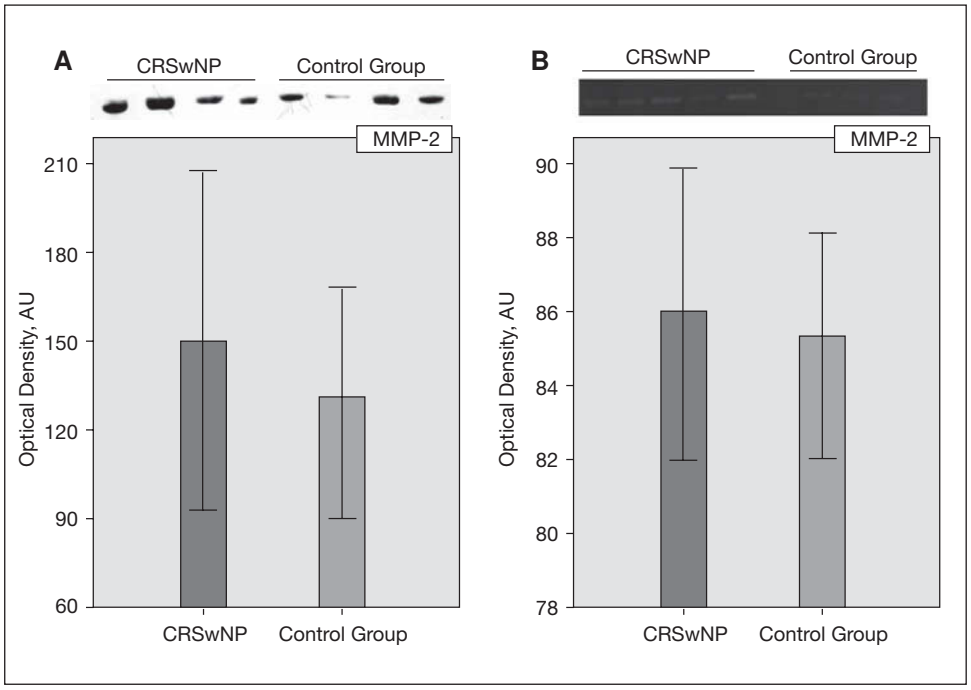

Figure 2. Western blot (A) and zymography (B) results for MMP-2. AU indicates arbitrary units; CRSwNP, chronic rhinosinusitis with nasal polyposis. *Null hypothesis: median test, *P*<.05.

With this paper we have tried to understand the involvement of MMP-9 in CRSwNP, not only in terms of its expression, but also the activity of this isoform using zymography. In the same way, the still somewhat doubtful role of MMP-2 in this condition has been studied.

Our results showed an increase in MMP-9 expression and activity in the samples obtained from patients compared with the control samples. However, no differences were found in the study of MMP-2.

In conclusion, our results highlight the importance of MMP-9 in the physiopathology of this illness, which might explain, at least in part, the histological remodelling typical of nasal polyps. At the same time, our data indicate that MMP-9 is a possible therapeutic target for this illness

and there is a clear lack of relevance for MMP-2 in CRSwNP.

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