Obestatin does not modulate:

- Cell cycle
- LDH levels
- Alkaline phosphatase
- Fatty acid uptake
- Glucose uptake
- Matrix metalloproteases 3 and 9
- Nitric Oxidase synthase type2

Figure 1 Obestatin inhibits chondrocyte metabolic activity, evaluated by the MTT colorimetric method, in a dose-dependent manner. *** $p < 0.001$. As a positive control, metabolic activity was evaluated in the presence of 5% (vol/vol) fetal bovine serum (FBS). LDH, Lactate dehydrogenase.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Dr Juan Viñuela and Manuel Otero, BS (Santiago University Clinical Hospital, Central Laboratory of Biochemistry and Immunology) for their contribution to this work.

Authors' affiliations

Rocio Lago, Rodolfo Gomez, Juan J Gomez-Reino, Oreste Gualillo, Santiago University Clinical Hospital, Research Laboratory 4 (NEIRID LAB, Laboratory of Neuro Endocrine Interactions in Rheumatology and Inflammatory Diseases), Santiago de Compostela, Spain

Carlos Dieguez, University of Santiago de Compostela, Department of Physiology, School of Medicine, Santiago de Compostela, Spain

Francisca Lago, Santiago University Clinical Hospital, Research Laboratory 1 (Molecular and Cellular Cardiology), Santiago de Compostela, Spain

The research described in this article has been supported by the Spanish Ministry of Health, Fondo de Investigación Sanitaria, Instituto de Salud Carlos III (FIS PI 05/0525, PI030115, PI050419) and Xunta de Galicia. O.G. and F.L. are recipients of a contract under the "Programme of stabilization of researchers" co-funded by the Instituto de Salud Carlos III and SERGAS. R.L. and R.G. are recipients of a project-associated fellowship from Instituto de Salud Carlos III.

Correspondence to: Oreste Gualillo, Santiago University Clinical Hospital, Area, NEIRID Laboratory 4, Trav. Choupana sn, 15706 Santiago de Compostela, Spain; gualillo@usc.es

Accepted 29 March 2007

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Endothelial nitric oxide synthase +894 polymorphism is associated with recurrent salivary gland swelling and early onset in patients with primary Sjögren's syndrome

Marja Pertovaara, Jaakko Anttonen, Mikko Hurme

Ann Rheum Dis 2007;**66**:1400–1401. doi: 10.1136/ard.2007.069385

Primary Sjögren's syndrome (SS) is a chronic autoimmune disease that affects primarily the salivary and lacrimal glands. Nitric oxide (NO) has been implicated in the pathogenesis of SS and raised nitrite concentrations have been observed in the saliva of patients with SS.¹ NO is a molecule that plays a key role in many physiological and pathological processes. It is produced *in vivo* from the amino acid L-arginine by a family of nitric oxide synthases (NOS), of which three isoforms have been identified, i.e. neuronal, endothelial and inducible.² Endothelial nitric oxide synthase (eNOS) is a constitutively expressed isoform of NOS. The eNOS gene entails

several polymorphisms, of which for example the +894 T>G polymorphism is of functional significance, the T allele having been associated with high NO production.³

We sought to establish whether the eNOS +894 polymorphism is associated with susceptibility to primary SS or to the salivary manifestations of the disease, and after informed consent analysed the genetic polymorphism of eNOS (+894T>G, rs1799983) in 64 (62 female, two male) patients

Abbreviations: eNOS, Endothelial nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; SS, Sjögren's syndrome

Table 1 Demographic and clinical characteristics of patients with primary Sjögren's syndrome carrying and not carrying allele T of endothelial nitric oxide synthase +894

	eNOS +894 allele T		
	Carriers (n = 30)	Non-carriers (n = 34)	p
Women : men	30 : 0	32 : 2	
Age, years (IQR)	59 (50–65)	63 (56–69)	0.150
Age at disease onset, years (IQR)	48 (39–54)	57 (47–60)	0.009
Disease duration, years (IQR)	10 (7–13)	8 (4–11)	0.015
Sicca symptoms of the eyes	26 (90%)*	29 (85%)	0.604
Sicca symptoms of the mouth	28 (97%)*	32 (94%)	0.651
Recurrent salivary gland swelling	18 (62%)*	11 (32%)	0.018
Histological grade in LSG ≥ 3	23 (77%)	24 (71%)	0.583
Antinuclear antibody positivity	23 (79%)*	31 (91%)	0.180
Anti-SSA antibody positivity	21 (72%)*	23/33 (70%)	0.814
Anti-SSB antibody positivity	16 (55%)*	17/33 (52%)	0.773

eNOS, Endothelial nitric oxide synthase; pSS, primary Sjögren's syndrome; IQR, interquartile range; LSG, labial salivary gland biopsy.

*n = 29.

Statistics: Mann-Whitney U test and chi-square test.

with primary SS fulfilling three or more modified Californian criteria⁴ (histological findings were graded on the Chisholm-Mason scale,⁵ grades 3 and 4 being regarded as diagnostic) and in 400 healthy blood donor controls. The genotyping was performed by the 5'nucleotidase method (TaqMan, Applied Biosystems, Foster City, California, USA). A careful clinical examination and interview had previously been conducted with these patients.⁶ The study protocol was approved by the Ethical Committee of Tampere University Hospital. The genotype distributions followed the Hardy-Weinberg equilibrium.

The eNOS genotype or allele frequencies between primary SS patients (GG 53%, GT 41%, TT 6%) and controls (GG 57%, GT 35%, TT 8%) did not differ ($p = 0.642$, chi-square test).

The primary SS patients carrying the high NO producer allele T of the eNOS +894 had a history of parotid or submandibular gland swelling significantly more frequently than non-carriers, and age at disease onset was significantly lower in T allele carriers compared with others (table 1). The presence of extraglandular manifestations of primary SS did not differ between the groups (data not shown).

There are previous data on the role of NO in various oral diseases,² but the observed association of eNOS polymorphism with effects on the salivary gland has not previously been reported. Immunohistochemical studies have demonstrated the presence of eNOS in ductal epithelial cells in normal human salivary glands.⁷

Salivary gland swelling has previously been associated with the early onset of primary SS.⁸ Our present results on the effects of the eNOS polymorphism on both age at disease onset and recurrence of salivary gland swellings would be in line with the concept of a persistent salivary gland infection in the pathogenesis of primary SS.⁹ The association between eNOS genetics and age at onset of primary SS could be explained by a possible influence of the high producer genotype on the timepoint of a viral infection triggering the pathogenesis of primary SS. NO has been shown to play a physiological role in human B-cell biology by inhibiting programmed cell death and maintaining Epstein-Barr virus latency.¹⁰

ACKNOWLEDGEMENTS

The authors would like to thank Ms Sinikka Repo-Koskinen and Ms Eija Spåre for skilful technical assistance.

Authors' affiliations

Marja Pertovaara, Department of Internal Medicine, Section of Rheumatology, Tampere University Hospital, Tampere, Finland

Jaakko Anttonen, Department of Internal Medicine, Tampere University Hospital, Tampere, Finland

Mikko Hurme, Department of Microbiology and Immunology, University of Tampere, Medical School, Tampere, Finland

This study was supported by the Medical Research Fund of Tampere University Hospital, Tampere, Finland.

Correspondence to: Dr Marja Pertovaara, Department of Internal Medicine, Section of Rheumatology, Tampere University Hospital, PO Box 2000, FIN-33521 Tampere, Finland; marja.pertovaara@uta.fi

Accepted 14 April 2007

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