

Serum Relaxin in Systemic Sclerosis

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ABSTRACT. Objective. To evaluate serum levels of relaxin (RLX), a hormone with acknowledged antifibrotic activity, in patients with systemic sclerosis (SSc).

Methods. We performed a pilot study of 50 outpatients with SSc and 50 healthy subjects. Serum RLX was measured using the relaxin ELISA. Statistical analysis was performed using Student's *t* test.

Results. Serum RLX appeared to be significantly higher ($p < 0.001$) in patients with SSc compared to controls. RLX appeared significantly increased ($p < 0.001$) in male patients compared to male controls, and in female patients compared to female controls. RLX was significantly higher ($p < 0.001$) in female patients and female controls compared to male patients and male controls.

Conclusion. In patients with SSc, the increased level of RLX represents a defensive response against the fibrotic process. (J Rheumatol 2005;32:2164–6)

Key Indexing Terms:
SCLERODERMA

SYSTEMIC SCLEROSIS

RELAXIN

The peptide hormone relaxin (RLX) was discovered in 1926 when Hisaw performed experiments in female nonpregnant guinea pigs¹. Later, RLX was found to promote growth of the mammary gland, inhibit uterine contractile activity, and dilate and soften the cervix uteri². RLX is now considered a hormone with more than reproductive functions: it has antifibrotic, vasodilator, and pro-angiogenic properties; it controls pituitary hormone release, interacts with cerebral cortical receptors, induces the second phase of testicular descent, and increases ovarian apoptosis^{3,4}. The recent discovery of high-affinity saturable binding sites that bind RLX in many tissues (like atria, cerebral cortex, hypothalamus, gonads, etc.³⁻⁵) confirmed its pleiotropic activity. Regarding antifibrotic effects more specifically, RLX has been reported to reduce fibrosis in the skin, kidney, heart, lung, and liver, acting on collagen and extracellular matrix remodeling by promoting a decrease in collagen production and an increase in expression of metalloproteinases (MMP)³. In particular, recombinant human RLX acts directly on several fibroblast cell lines to promote both a decrease in the expression of mRNA encoding type I and type III collagen and synthesis of the proteins, together with an increase in pro-MMP expression. In addition, recombinant human

RLX has been used to decrease collagen accumulation in several rodent models of induced fibrosis, including a mouse model of lung fibrosis, and rat models of renal and liver fibrosis⁵. Casten and Boucek⁶ reported that daily intramuscular injection of partially purified P1 RLX over a period of 6–30 months favorably influenced 3 features of SSc: skin tightness, Raynaud's phenomenon, and trophic ulcers. Subsequently, the antifibrotic activity of the hormone induced some researchers^{7,8} to utilize RLX in the treatment of SSc, a connective tissue disease in which tissue fibrosis is the predominant pathogenetic and clinical feature, but there are few studies and the results are still under debate. In particular, Erikson and Unemori⁸ did not corroborate the efficacy of RLX previously reported by Seibold and coworkers⁷. Moreover, we noted that there are no studies on serum levels of RLX in patients with SSc, and we felt that this information might contribute to understanding the potential of RLX for treatment of SSc.

MATERIALS AND METHODS

Study participants. We performed an open pilot study of 50 outpatients with SSc (mean age \pm standard deviation, SD: 60 ± 3.9 yrs) diagnosed according to the 1980 American Rheumatology Association diagnostic and therapeutic criteria⁹. All patients [41 women (18 of reproductive age and 23 menopausal) and 9 men] were enrolled among those followed in our department in the last 5 years. Mean duration of disease was 4.0 months \pm 1.4 years. Disease severity was determined according to LeRoy and coworkers' criteria¹⁰: in particular, 32 patients (64%) had limited SSc and 18 (36%) had diffuse disease. All patients were receiving calcium channel blockers (nifedipine) and 40 (80%) were receiving D-penicillamine. Other drugs, such as angiotensin-converting enzyme inhibitors, low-dose oral prednisone, nonsteroidal antiinflammatory drugs, proton pump inhibitors, or prokinetic agents, were administered at the physician's discretion or on the basis of visceral involvement. Patients were compared with 50 age and sex matched controls [41 women (18 of reproductive age and 23 menopausal) and 9 men, mean age 65 ± 4.3 yrs] from the medical and nursing staff of our hospital. Patients and controls, correctly informed about the study plan,

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gave their written consent. In all subjects, serum RLX was estimated using a validated¹¹⁻¹³ Relaxin Elisa commercial kit (Immunodiagnostik AG, Bensheim, Germany). This kit measures H1 and H2 RLX and appears to be specific for the hormone as it does not show any cross-reactivity with insulin and insulin-like growth factors.

Statistical analysis. Results were analyzed using the Student t test.

RESULTS

Table 1 describes the serum RLX levels in the total population of patients and controls, and in subgroups of patients and controls divided on the basis of sex and presence or absence of menstruation. Figure 1 compares the serum RLX values in patients and controls, controlling for sex and presence or absence of menstruation. Our data show that serum RLX values were significantly higher ($p < 0.001$) in patients with SSc compared to controls. Moreover, serum RLX appeared significantly increased ($p < 0.001$) in male patients compared to male controls, and in female patients compared to female controls. Further, serum RLX was significantly higher ($p < 0.001$) in female patients and controls compared to male patients and controls. No statistical difference was found in serum RLX among female patients and female con-

trols, whether they were of reproductive age or in menopause.

DISCUSSION

As reported by others^{2-4,12}, we confirm that RLX is not only strongly linked to female reproductive functions. Indeed, the hormone is present in both sexes, and serum RLX levels in women seem unaffected by age. This finding emphasizes the pleiotropic role of the hormone, which acts in different physiological and pathological conditions. Antifibrotic properties of the hormone justify its therapeutic use in SSc. Moreover, despite debate regarding the efficacy of RLX for SSc, RLX treatment continues to be reported^{14,15}.

There are no previous studies on the level of serum RLX in patients with SSc, while the hormone has been evaluated in some other pathological conditions^{16,17}. We describe the increase of serum RLX in our patients with SSc compared to controls. On the basis of our results, even if we exclude the possibility of a causal interrelationship between serum RLX and SSc, we suggest the following hypotheses: (1) RLX overproduction and secretion might represent a defen-

Table 1. Serum RLX levels (pg/ml) in patients with SSc and controls. Results are expressed as mean \pm SD. All groups and subgroups were statistically significantly different from each other ($p < 0.001$) with the exception of female patients of reproductive age versus menopausal patients (NS) and female controls of reproductive age versus menopausal controls (NS).

	Patients			Controls		p
	n (%)	Serum RLX		n (%)	Serum RLX	
All	50 (100)	19.0 \pm 3.3	All	50 (100)	13.9 \pm 2.8	< 0.001
Men	9 (18)	13.3 \pm 1.2	Men	9 (18)	8.3 \pm 0.8	< 0.001
Women	41 (82)	23.3 \pm 2.0	Women	41 (82)	15.0 \pm 1.1	< 0.001
Reproductive age	18 (43.9)	20.4 \pm 2.2	Reproductive age	18 (43.9)	15.3 \pm 1.3	< 0.001
Menopausal	23 (56.1)	20.2 \pm 1.9	Menopausal	23 (56.1)	14.9 \pm 0.9	< 0.001

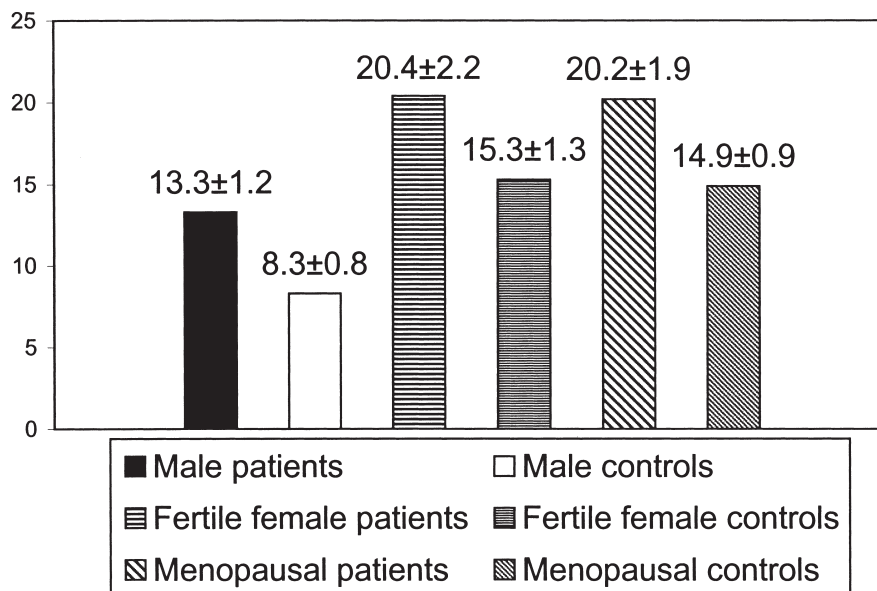


Figure 1. Mean (\pm SD) serum RLX levels (pg/ml) in patients and controls.

sive response against the fibrotic process, due to the well known antifibrotic activities of the hormone³; and (2) Serum RLX increase in patients with SSc might be due to an anatomic and/or functional deficiency of specific tissue receptors. Both these hypotheses need further investigation, in particular the second one, because there are no studies on the presence of RLX receptors in dermal connective tissue. Another open question is the source of RLX in patients with SSc. Recently this was raised by Binder and coworkers¹⁶, who found elevated concentrations of serum RLX in patients with metastatic breast cancer. If the tumor is the source of RLX, could tissue in SSc not act the same way? In this case, RLX serum levels would represent a serological marker of disease, and correlate with the degree of sclerosis. If this last hypothesis is correct, would RLX treatment be justified in this connective tissue disorder?

We propose a multicenter study on the possible interrelationship between RLX and SSc. Such a study would not only enable a larger sample of clinical cases (men in particular), but also provide an opportunity to correlate serum RLX levels with age and disease severity.

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