

Lymphocytic prolactin does not contribute to systemic lupus erythematosus hyperprolactinemia

D.B. Paraiba¹, C.R.J. Soares², P. Bartolini², F.S. Arthuso², E.F. Borba³,
E. Bonfa³, M.D. Bronstein¹

¹Neuroendocrine Unit, Division of Endocrinology and Metabolism, Hospital das Clínicas, University of São Paulo Medical School, São Paulo, Brazil; ²Biotechnology Department, Instituto de Pesquisas Energéticas e Nucleares – Comissão Nacional de Energia Nuclear, São Paulo, Brazil; ³Rheumatology Division, Hospital das Clínicas, University of São Paulo Medical School, São Paulo, Brazil.

Abstract

Introduction

Lymphocytic prolactin (PRL) gene expression is detected in the majority of the immune cells and it is not known if this source contributes to hyperprolactinemia in systemic lupus erythematosus (SLE). We have therefore evaluated lymphocytic PRL secretion and gene expression in SLE and healthy controls.

Methods

Thirty SLE patients (ACR criteria) and 10 controls were selected for the study. Serum levels of PRL and macroprolactin were detected by immunofluorometric assay and gel filtration chromatography, respectively. The lymphocytic biological activity was determined by Nb2 cells bioassays. Lymphocytic PRL gene expression was evaluated by RT-PCR assay.

Results

The median serum PRL levels of the 30 SLE patients was higher than the control group (9.65 (1.9–38.9) vs. 6.40 (2.4–10.3) ng/mL, $p=0.03$). A significant difference was detected between median serum PRL levels of active SLE, inactive SLE and controls (10.85 (5–38.9) vs. 7.65 (1.9–15.5) vs. 6.40 (2.4–10.3) ng/mL, $p=0.01$). The higher frequency of mild hyperprolactinemia was detected among active SLE in comparison with inactive SLE and controls (7(38.9%) vs. 1 (8.3%) vs. 0(0%)), with statistical significance ($p=0.02$). Nb2 cells assay revealed uniformly low levels of lymphocytic PRL in active, inactive and control groups without statistical significance among them (24.2 (8–63) vs. 27 (13.6–82) vs. 29.5 (8–72) ng/mL, $p=0.84$). Furthermore, median lymphocytic PRL gene expression evaluated by RT-PCR assay was comparable in both active and inactive SLE groups ($p=0.12$).

Conclusion

This is the first study to exclude a lymphocytic source of PRL, pointing out a pituitary etiology for hyperprolactinemia in SLE. However, other sources from the immune system cannot be ruled out.

Key words

prolactin, lupus erythematosus, hyperprolactinemia, bioassays, RT-PCR

Diane B. Paraiba, MD, PhD
 Carlos R.J. Soares, PhD
 Paolo Bartolini, PhD
 Fernanda S. Arthuso
 Eduardo F. Borba, MD, PhD
 Eloisa Bonfa, MD, PhD
 Marcello D. Bronstein, MD, PhD

This work was supported by FAPESP - 2005/51806-9, 2005/51805-2, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ grants no. 303165/2008-1 to EFB and no.305468/2006-5 to EB) and Federico Foundation Grant (to EFB and EB).

Please address correspondence and reprint requests to:

Marcello D. Bronstein, MD,
 Neuroendocrine Unit, Division of
 Endocrinology and Metabolism,
 Hospital das Clinicas,
 University of Sao Paulo Medical School,
 Avenida 9 de Julho, 3858 Jardins CEP,
 01406-100 São Paulo SP, Brazil.
 E-mail: mdbronstein@uol.com.br

Received on May 29, 2010; accepted in revised form on September 13, 2010.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2010.

Introduction

Prolactin (PRL) is known to have a double activity, functioning not only as a hormone but also as a cytokine. Human prolactin (PRL) consists of three main isoforms: monomeric (23KDa, considered to be the biological active molecule), dimeric ("big prolactin", bPRL ~50Kda) and macroprolactin ("big big prolactin", bbPRL >100Kda)

In fact, PRL gene expression can be determined in many sites including the majority of immune cells where it probably acts as a cytokine. In this regard, previous studies have suggested the role of PRL as an immunomodulator, but the precise contribution of this "cytokine-hormone" in the complex immune system is not completely understood (1-3). Data from experimental animal models and some clinical trials suggest that increased PRL levels could be related to autoimmune diseases (4-7). Indeed, the prevalence of mild hyperprolactinemia in systemic lupus erythematosus (SLE) is around 20–30% and it was demonstrated that high PRL levels may or may not be associated to disease activity (SLE) (8-20).

These data raise the hypothesis that PRL could be involved in the pathogenesis of autoimmune diseases, mainly by local lymphocyte production (12). As a matter of fact, lymphocytic PRL gene expression is found in the majority of the immune cells, where it acts as a cytokine, via paracrine and autocrine regulation (3) reinforcing the possible contribution of a lymphocytic source in SLE.

We therefore evaluated lymphocytic PRL secretion and its biological activity and PRL gene expression in patients with SLE.

Patients and methods

Patients

Thirty consecutive SLE patients were selected among our lupus patients attending the outpatient clinics of the Rheumatology Division of the University of São Paulo. All of them fulfilled 4 or more of the revised American College of Rheumatology criteria for the classification of SLE (21, 22). Exclusion criteria were presence of physiologic, pharmacologic or pathologic causes of hyperprolactinemia such as pregnancy, prolactinoma,

hypothyroidism, renal failure and PRL raising drugs. Ten healthy controls with the same exclusion criteria were selected for comparison. SLE manifestations included cutaneous and/or articular involvement, neuropsychiatric disease, renal disease, cardiopulmonary disease, and haematologic complications. Clinical activity was measured according to the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (23) and were arbitrarily divided into active (SLEDAI ≥ 4) and inactive (SLEDAI <4) disease. Most patients were on medical treatment for SLE, taking one or more drugs (chloroquine, mycophenolatemofetil, azathioprine, methotrexate, prednisone) Regarding the glucocorticoid dose, one patient was on 60mg/day, three patients were on 40mg/day, four patients on 30mg/day, twelve patients from 2.5 to 25mg/day, and the remaining 10 were not taking glucocorticoid at all.

The study was approved by the local Ethics Committee and informed consent was obtained from all participants.

Methods

-PRL assay

Serum PRL was quantitatively determined between 08:00h and 10:00h to avoid variation due to circadian rhythms. Serum was kept frozen (-20°C) for hormone assays which were performed by an immunofluorometric assay (Auto DELFIA Prolactin Perkin Elmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). The normal reference value is up to 14.5ng/mL. Intra and inter-assay coefficients of variation were 1.2 and 3.1%, respectively.

-Macroprolactinemia assessment

Macroprolactinemia was investigated in all hyperprolactinemic patients by polyethylene glycol (PEG) precipitation (24), and confirmed by gel-filtration chromatography, using a column of 1.6x30cm Superdex 200 (Pharmacia, Uppsala, Sweden), eluted by FPLC with 20mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, 15mM NaCl, and 10mg/liter of gentamicin (pH 7.5).

-Cell preparation and cultures

Peripheral blood mononuclear cells (PBMNC) were obtained by Ficoll

Competing interests: none declared.

gradient centrifugation (Ficoll-Paque®, GE Healthcare) (25, 26), with slight modifications. PBMNC were recovered from the interface and cultured at a concentration of 2.0×10^6 cells/mL in serum-free culture medium AIM-V (Gibco™ AIMV Medium liquid). This technique yields up to 95% of lymphocytes. Viability of cells, as determined by Trypan blue exclusion, was always above 95%. Cells were incubated for 72 hours at 37°C and 5% CO₂. Culture media were separated from cells by centrifugation at 1000g and kept at 80°C until it was analysed for PRL bioactivity, while cells were prepared for RNA extraction.

-Bioassays

Nb2 bioassay

Cells were routinely maintained as suspension cultures in RPMI 1640 medium supplemented with 10% horse serum, 10% foetal bovine serum, 50U/ml penicillin, 50µg/ml streptomycin (Invitrogen, Carlsbad, CA), and 0.1mM β-mercaptoethanol. The Nb2 bioassay was performed as described by Tanaka *et al.* (27), with some modifications: the cells were prepared immediately before assay and diluted in colourless RPMI. The cells were incubated at 37°C and 5%CO₂. Cell number was assessed 72h after plating by MTS (3(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H- Tetrazolium-minner salt) (Promega Corp., Madison, WI) assay. MTS dye at 2mg/ml in PBS was mixed at a 20:1 ratio (vol/vol) with phenazinemethosulfate (Sigma, St. Louis, MO), 0.92mg/ml in PBS. Twenty microfibrils of the mixture were then added to each well, and kept there for 2h after incubation at 37°C, before reading the absorbance at 490nm in a microplate reader (model MR4000; Dynatech, Chantilly, VA).

The recombinant PRL (rPRL) preparation (97/714) of Human PRL WHO was used as reference in bioassays. This rPRL was diluted in the same lymphocytes culture medium (AIM V). A standard curve with rPRL was constructed in each assay. The bioactivity and lymphocytic PRL level for each case was calculated according to a standard curve.

Ba/F-LLP bioassay

Ba/F-LLP cells are derived from Ba/F-3 (murine pro-B cells dependent on IL-3 for growth; they were previously transfected with a plasmid encoding the long isoform of the human PRLR) and depend on PRL for their growth (28, 29). Cells were routinely maintained in suspension in RPMI 1640 medium supplemented with 10% heat-inactivated foetal calf serum; 2mM glutamine; 50U/ml penicillin; 50µg/ml streptomycin; 700µg/ml geneticin, and 1µg/liter rPRL. Before being submitted to proliferation assay, cells were starved for 6h in 1% foetal calf serum colourless RPMI 1640 medium with additives and then distributed in a flat-bottom 96-well plate at a density of 2.5×10^4 cells/well and in a final volume of 200µl. After 72h at 37°C and 5% CO₂, the number of viable cells was assessed using the MTS assay.

The same recombinant PRL (rPRL) preparation (97/714) of Human PRL WHO was used as reference in Nb2 bioassay. This rPRL was diluted in the same medium as lymphocyte cultures (AIM V). A standard curve with rPRL was constructed in each assay. The bioactivity and lymphocytic PRL level for each case was calculated with basis on the standard curve. These procedures were similar by carried out for Nb2 bioassays.

-Quantitative RT-PCR

After culture, total lymphocyte RNA was isolated from the SLE and control groups, the latter forming a pool of normal control RNA, through guanidiniumthiocyanate-chloroform extraction (Trizol Reagent, Invitrogen, Carlsbad, CA) (30).

Specific exon-primers for PRL and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Table I) used as an internal endogenous control were designed using the software Primer3 (<http://frodo.wi.mit.edu/primer3/>).

Real time RT PCR was carried out in a fluorometric thermal cycle (ABI Prism 7000 Sequence Detection System). Fluorescence was monitored during every RT PCR cycle at the annealing step.

Real-time RT-PCR was performed in a single step method (31), using Quantitect SYBR® Green RT PCR kit (Qiagen; GmbH, D Hilden). Reverse transcription was performed at 50°C for 5 minutes, followed by 5 minutes at 95°C. After that time, the reverse transcriptase is destroyed and DNA polymerase is activated. Reactions were performed in a 25µL volume with 150ng RNA; 0.5nM forward and reverse primers for the housekeeping gene (GAPDH) and for the PRL gene and 2x Quant Text Syber Green and Quant Text RT mix (Qiagen; GmbH, D Hilden). Forty RT-PCR cycles (20 seconds at 95°C, 30 seconds at 55°C, 30 seconds at 72°C) were run in an ABI PRISM 7000 rotor.

All Real-time RT-PCR assays were performed in triplicate on material from the same RNA and its counterpart; no RT mRNA samples were included as negative control and a normal pool of control group was included as positive control. The internal endogenous control (GAPDH) sample was included in every plate. PCR efficiency was examined by serially diluting the template cDNA for a pool of normal RNA of lymphocytes; at the end of the PCR, a melting curve analysis was performed by gradually increasing the temperature from 65°C to 95°C (0.1°C/sec) to confirm amplification specificity of the PCR products.

The relationship between the threshold cycle (Ct) and the logarithm of RNA concentration was studied for determining the correlation coefficient and the slope. The Ct indicates the fractional number at which the amount of amplified target reaches a fixed threshold (32, 33).

Table I. exon-primers (PRL and GAPDH).

	Sense	Anti-sense
PRL	5' GAAGTGTGTTTCTCTGCAACG 3'	5' GGTCAAACAGGTCTCGAAGG 3'
GAPDH	5' CGCTCTCTGCTCCTCTGTT 3'	5' TGAGGTCAATGAAGGGGTCA 3'

The $2^{-\Delta\Delta C_t}$ method was used to calculate the relative expression of lymphocytic PRL gene for active and inactive SLE (34).

The quality of individual pairs of primers was confirmed by real-time PCR analysis followed by agarose gel electrophoresis to ensure that there was only one product of the expected size and no detectable primer-dimer formation.

Statistical analysis

Data are presented as median and range. Statistic analyses were made using the Mann-Whitney and Kruskal-Wallis methods. Spermann method used for correlations. Statistical significance was set at $p < 0.05$.

Results

Serum PRL assay

The median age (32 (20–48) vs. 31.5 (24–42) years, $p=0.97$) and female gender predominance (90% vs. 90%, $p=1.0$) were similar in SLE patients and controls. The median serum PRL level of the 30 SLE patients was higher than the control group (9.65 (1.9–38.9) vs. 6.40 (2.4–10.3) ng/mL, $p=0.03$). Mild hyperprolactinemia (<50 ng/mL) was detected in 8 patients and none of the controls (8 vs. 0, $p=0.16$). Macroprolactinemia was confirmed by gel-filtration chromatography in one patient. Levels of monomeric PRL were similar in SLE and healthy individuals (9.35 (1.9–38.9) vs. 6.4 (2.4–10.3) ng/mL, $p=0.06$).

Active disease was identified in 18 patients (60%) and inactive disease in 12 (40%), with a median SLEDAI of 8.5 (4–23) and 0, respectively. Frequencies of lupus manifestations were similar between SLE patients with active and inactive disease: cutaneous manifestations (100% vs. 75%, $p=0.10$), joint involvement (94.4% vs. 91.7%, $p=0.65$), neuropsychiatric disease (22.2% vs. 16.7%, $p=0.92$), renal disease (38.9% vs. 33.3%, $p=0.93$), cardiopulmonary involvement (22.2% vs. 41.7%, $p=0.46$), and hematologic complications (50% vs. 33.3%, $p=0.59$). Significant difference was detected among median serum PRL levels of active SLE in comparison with inactive SLE and controls (10.85 (5–38.9)

Table II. Laboratorial findings in active/inactive SLE patients and controls.

	Active SLE	Inactive SLE	Control	<i>p</i> -value
	<i>n</i> =18	<i>n</i> =12	<i>n</i> =10	–
Serum PRL (ng/mL)	10.85 (5–38.9)	7.65 (1.9–15.5)	6.4 (2.4–10.3)	0.01
Hyperprolactinemia (%)	7 (38.8%)	1 (8.33%)	0	0.02
Macroprolactinemia (%)	0 (0%)	1 (8.33%)	0 (0%)	Not applicable
Monomeric PRL (ng/mL)	10.85 (5–38.9)	7.1 (1.9–12.3)	6.4 (2.4–10.3)	0.06
Lymphocytic PRL (ng/mL)	24.2 (8–63)	27 (13.6–82)	29.5 (8–72)	0.84

vs. 7.65 (1.9–15.5) vs. 6.40 (2.4–10.3) ng/mL, $p=0.01$). The higher frequency of mild hyperprolactinemia was detected among active SLE, inactive SLE and controls (7 (38.9%) x 1 (8.3%) x 0 (0%)), with statistical significance ($p=0.02$). (Table II). Nevertheless, no correlations were detected between SLE manifestation and PRL level.

Bioassays and Quantitative RT-PCR

Nb2 cells assay revealed a uniformly low levels of lymphocytic PRL in active, inactive and control groups without statistical significance amongst them (24.2 (8–63) vs. 27 (13.6–82) vs. 29.5 (8–72) ng/mL, $p=0.84$) (Fig. 1). There was no correlation between glucocorticoid treatment (independent of the doses employed) and the level of lymphocytic PRL ($r=-0.44$, $p=0.10$). Furthermore, median lymphocytic PRL gene expression evaluated by RT-PCR assay was comparable in both active and inactive SLE groups ($p=0.12$) (Fig. 2).

Of note, a positive correlation was found between lymphocytic PRL gene expression and lymphocytic PRL bioactivity in Nb2 assay, showing that lymphocytic PRL production is proportional to its bioactivity (Fig. 3).

In our study, Ba/F-LLP cells assay was performed in all patients and controls; however the values obtained were below the sensitivity of the method and therefore not reliable for evaluating lymphocytic PRL (data not shown).

Discussion

This study suggests that lymphocytic PRL does not contribute to hyperprolactinemia of SLE, supporting a pituitary origin for PRL hypersecretion.

In the last 10 years, there has been a growing body of evidence that strongly supports a close link between hyperprolactinemia and autoimmunity. In fact, most of the studies in SLE reinforce this possibility since hyperprolactinemia was described in about 20%–30% of lupus patients, usually with mildly

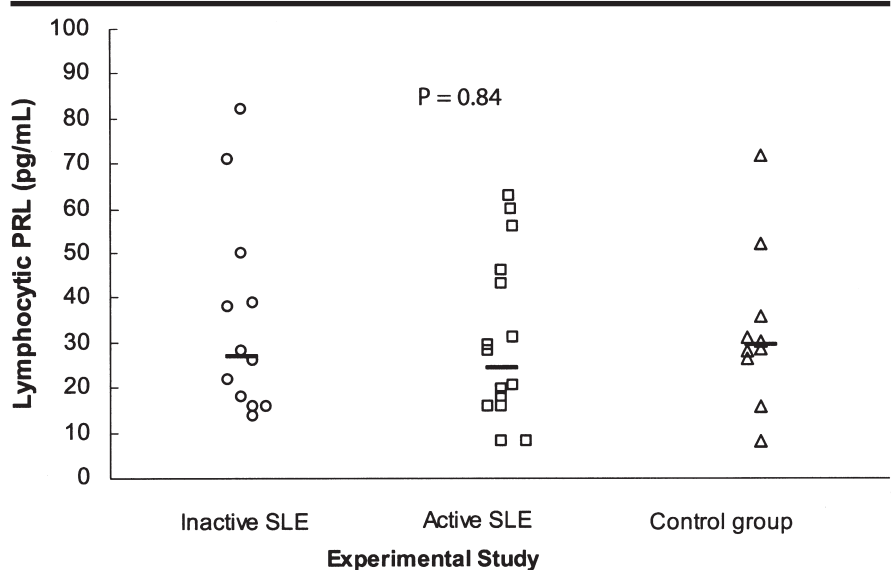


Fig. 1. Median lymphocytic PRL levels in active ($n=18$) and inactive ($n=12$) SLE patients and controls ($n=10$) as determined by Nb2 cell assay.

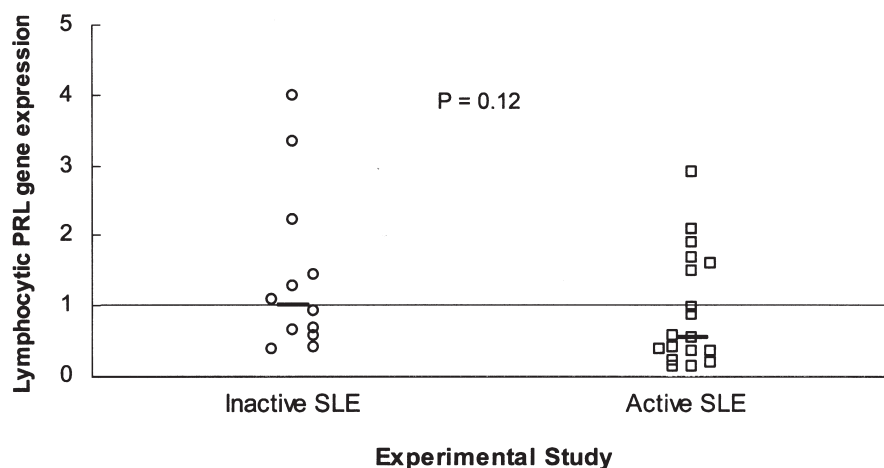


Fig. 2. Median lymphocytic PRL gene expression in active (n=18) and inactive (n=12) SLE patients. Control group is represented by a lymphocytic PRL gene expression =1 (horizontal line).

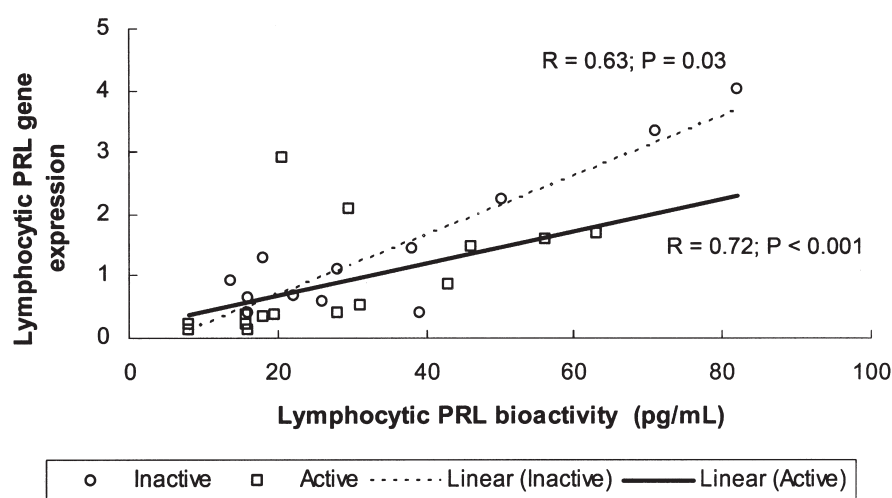


Fig. 3. Correlation between lymphocytic PRL levels and its gene expression in active (n=18) and inactive (n=12) SLE patients.

elevated PRL levels (13), as also observed in our study.

Importantly, a positive relationship between hyperprolactinemia and disease activity has been demonstrated in some studies, but not confirmed by others (17). Our data support that PRL levels are influenced by the inflammatory status of the disease. Some of the previous association may be explained by different methods of measuring disease activity and more importantly, the use of PRL assays that did not exclude macroprolactin (bbPRL) (14, 35, 36). This latter isoform (bbPRL) shows less *in vivo* and *in vitro* bioactivity as compared to monomeric PRL (29), and in SLE it was suggested to be a possible "protective factor" (35, 37). The estimated prevalence of macroprolactine-

mia in subjects with normal PRL levels is 0.15%–1.3% in contrast to 15.4%–46% in subjects with hyperprolactinemia(38). The low prevalence in our SLE patients supports the notion that macroprolactinemia is not an autoimmune phenomenon (39).

Another important issue of the present study was to define the origin of hyperprolactinemia in SLE. Some studies pointed out the possibility of an extrapituitary source but this is still a matter of debate. Nb2 assay used herein has the advantage to be extremely sensitive and able to detect minimum amounts of PRL, such as 8pg/mL or less and should therefore be considered a reliable measurement. Ba/F-LLP assays would be of great help in clarifying some bioactivity differences among groups

since it is a homologous assay (28, 29). However, our bioassay with Ba/F-LLP cells revealed to be inadequate for the evaluation of lymphocytic PRL bioactivity due to its low sensitivity (data not shown). Actually, in the literature, Ba/F-LLP bioassay is described as less sensitive than Nb2 bioassay to evaluate serum PRL bioactivity (29).

The finding of low and comparable lymphocytic PRL levels in all studied groups suggests that lymphocyte is not directly implicated in SLE hyperprolactinemia. Furthermore, active and inactive SLE patients had similar lymphocytic PRL production which supports the notion that they are not associated with disease activity. In fact, secretion was too little to justify an increase in serum PRL level which is in accordance with the hypothesis of Ben-Jonathan *et al.* (40).

The possibility of a reduction in bioactivity due to the freezing and throwing of the samples (41) (supernatants of cultures) which would justify the low activity observed in all groups is unlikely. In this regard, similar lymphocytic PRL gene quantitative expressions (qRT-PCR) were detected in active and inactive SLE emphasising the consistency of the Nb2 assay findings. Additionally, there was a positive correlation between lymphocytic PRL gene expression and its bioactivity showing that transcript was translated into protein and that the determination of lymphocytic PRL from its bioactivity is, indeed, reliable. Moreover, qRT-PCR demonstrated similar expression of lymphocytic PRL gene in SLE regardless disease activity status.

Immunosuppressive therapy does not seem to account for our findings since previous studies have demonstrated that these drugs do not affect pituitary PRL levels (42–46). On the other hand, steroid therapy reduces pituitary PRL, an effect largely dose-dependent. This effect was minimised in the present study, since few patients were under high doses, and their inclusion in the analysis may have underestimated PRL serum levels in active SLE patients.

Previous studies have suggested good response with bromocriptine (a dopaminergic agonist) in lupus experi-

mental models (NZB/NZW) (47) and in active SLE patients (48, 49). The use of this drug would be an indirect way to show pituitary involvement lupus hyperprolactinemia since extrapituitary PRL is not controlled by dopaminergic tonus (50). The molecular and cellular experiments performed herein provided strong evidence to exclude the role of lymphocytic PRL in the etiology of high PRL levels in lupus. Other sources from the immune system, however, cannot be ruled out.

In conclusion, we have demonstrated by gene expression and bioactivity assays strong evidence of pituitary etiology for hyperprolactinemia in SLE. The role of lymphocytic PRL receptor expression needs to be further investigated in order to define if distinct lymphocyte sensitivity to this pituitary PRL enables a direct endocrine, paracrine or autocrine PRL actions in immune system cells.

Acknowledgements

We acknowledge the collaboration of researchers from Instituto de Pesquisas Energeticas e Nucleares – Comissão Nacional de Energia Nuclear (Cibele Nunes Peroni, Susana da Rocha Heller, Maria Teresa de Carvalho Pinto Ribela, Renata Damiani and Joao Ezequiel de Oliveira).

References

1. FREEMAN ME, KANYICKA B, LERANT A, NAGY G: Prolactin: Structure, Function, and Regulation of Secretion. *Physiol Rev* 2000; 80: 1523-631.
2. BOLE-FEYSOT C, GOFFIN V, EDERY M, BINART N, KELLY PA: Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev* 1998; 19: 225-68.
3. BEN-JONATHAN N, MERSHON JL, ALLEN DL, STEINMETZ RW: Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. *Endocr Rev* 1996; 17: 639-69.
4. WALKER SE, ALLEN SH, MCMURRAY RW: Prolactin and autoimmune disease. *Trends Endocrinol Metab* 1993; 4: 147-51.
5. ORBACH H, SHOENFELD Y: Hyperprolactinemia and autoimmune diseases. *Autoimmun Rev* 2007; 6: 537-42.
6. CHUANG E, MOLITCH ME: Prolactin and autoimmune diseases in humans. *Acta Biomed* 2007; 78 (Suppl. 1): 255-61.
7. DE BELLIS A, BIZZARRO A, PIVONELLO R, LOMBARDI G, BELLASTELLA A: Prolactin and autoimmunity. *Pituitary* 2005; 8: 25-30.
8. LAVALLE C, LOYO E, PANIAGUA R *et al.*: Correlation study between prolactin and androgens in male patients with systemic lupus erythematosus. *J Rheumatol* 1987; 14: 268-72.
9. JARA-QUEZADA L, GRAEF A, LAVALLE C: Prolactin and gonadal hormones during pregnancy in systemic lupus erythematosus. *J Rheumatol* 1991; 18: 349-53.
10. JARA LJ, PACHECO-REYES H, MEDINA G, ANGELES U, CRUZ-CRUZ P, SAAVEDRA MA: Prolactin levels are associated with lupus activity, lupus anticoagulant, and poor outcome in pregnancy. *Ann N Y Acad Sci* 2007; 1108: 218-26.
11. FOLOMEEV M, PROKAEVA T, NASSONOVA V, MASENKO E, OVTRAHT N: Prolactin levels in men with SLE and RA. *J Rheumatol* 1990; 17: 1569-70.
12. PEEVA E, VENKATESH J, MICHAEL D, DIAMOND B: Prolactin as a modulator of B cell function: implications for SLE. *Biomed Pharmacother* 2004; 58: 310-19.
13. JARA LJ, VERA-LASTRA O, MIRANDA JM, ALCALA M, ALVAREZ-NEMEGYEI J: Prolactin in human systemic lupus erythematosus. *Lupus* 2001; 10: 748-56.
14. VERA-LASTRA O, MENDEZ C, JARA LJ *et al.*: Correlation of prolactin serum concentrations with clinical activity and remission in patients with systemic lupus erythematosus. Effect of conventional treatment. *J Rheumatol* 2003; 30: 2140-6.
15. MOSZKORZOVÁ L, LACINOVÁ Z, MAREK J, MUSILOVÁ L, DOHNALOVÁ A, DOSTÁL C: Hyperprolactinaemia in patients with systemic lupus erythematosus. *Clin Exp Rheumatol* 2002; 20: 807-12.
16. PASOTO SG, MENDONÇA BB, BONFÁ E: Menstrual disturbances in patients with systemic lupus erythematosus without alkylating therapy: clinical, hormonal and therapeutic associations. *Lupus* 2002; 11: 175-80.
17. PACILIO M, MIGLIARESI S, MELI R, AMBROSONE L, BIGLIARDO B, DI CARLO R: Elevated bioactive prolactin levels in systemic lupus erythematosus - association with disease activity. *J Rheumatol* 2001; 28: 2216-21.
18. JACOBI AM, ROHDE W, VENTZ M, RIEMKASTEN G, BURMEISTER GR, HIEPE F: Enhanced serum prolactin (PRL) in patients with systemic lupus erythematosus: PRL levels are related to the disease activity. *Lupus* 2001; 10: 554-61.
19. JARA LJ, LAVALLE C, FRAGA A *et al.*: Prolactin, immunoregulation, and autoimmune diseases. *Semin Arthritis Rheum* 1991; 20: 273-84.
20. SHABANOVASS, ANANIEVALP, ALEKBEROVA ZS, GUZOV II: Ovarian function and disease activity in patients with systemic lupus erythematosus. *Clin Exp Rheumatol* 2008; 26: 436-41.
21. TAN EM, COHEN AS, FRIES JF *et al.*: The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25: 1271-1277.
22. HOCHBERG MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
23. BOMBARDIER C, GLADMAN DD, UROWITZ MB, CARON D, CHANG CH: Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35: 630-40.
24. VIEIRA JGH, TACHIBANA TT, OBARA LH, MACIEL RMB: Extensive experience and validation of polyethylene glycol precipitation as a screening method for macroprolactinemia. *Clin Chem* 1988; 44: 1758-9.
25. BÖYUM A: Isolation of leucocytes from human blood. Further observations. methylcellulose, dextran, and ficoll as erythrocyte aggregating agents. *Scand J Clin Lab Invest Suppl* 1968; 97: 31-50.
26. HARRIS R, UKAEJIOFO EO: Tissue typing using a routine one-step Lymphocyte separation procedure. *Br J Haematol* 1970; 18: 229-35.
27. TANAKA T, SHIU RPC, GOUT PW, BEER CT, NOBLE RL, FRIESEN HG: A new sensitive and specific bioassay for lactogenic hormones: measurement of prolactin and growth hormone in human serum. *J Clin Endocrinol Metab* 1980; 51: 1058-63.
28. BERNICHTEIN S, JEAY S, VAUDRY R, KELLY PA, GOFFIN V: New Homologous bioassays for human lactogens show that agonism or antagonism of various analogs is a function of assay sensitivity. *Endocrine* 2003; 20: 177-90.
29. GLEZER A, SOARES CR, VIEIRA JG *et al.*: Human macroprolactin displays low biological activity via its homologous receptor in a new sensitive bioassay. *J Clin Endocrinol Metab* 2006; 91: 1048-55.
30. CHOMCZYNSKI P, SACCHI N: Single-step method of RNA isolation by acid guanidiniumthiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; 162: 156-9.
31. WACKER MJ, GODARD MP: Analysis of one-step and two-step real-time RTPCR using SuperScript III. *J Biomol Tech* 2005; 16: 266-71.
32. GIULIETTI A, OVERBERGH L, VALCKX D, DECALONNE B, BOUILLON R, MATHIEU C: An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. *Methods* 2001; 25: 386-401.
33. GINZINGER DG: Gene quantification using real-time quantitative PCR: An emerging technology hits the mainstream. *Exp Hematol* 2002; 30: 503-12.
34. LIVAK KJ, SCHMITTGEN TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)). *Methods* 2001; 25: 402-8.
35. CRUZ J, AVINÁ-ZUBIETA A, DE LA ESCALERA GM, CLAPP C, LAVALLE C: Molecular heterogeneity of prolactin in the plasma of patients with systemic lupus erythematosus. *Arthritis Rheum* 2001; 44: 1331-5.
36. LEAÑOS-MIRANDA A, CHÁVEZ-RUEDA KA, BLANCO-FAVELA F: Biologic activity and plasma clearance of prolactin-IgG complex in patients with systemic lupus erythematosus. *Arthritis Rheum* 2001; 44: 866-75.
37. LEAÑOS-MIRANDA A, CÁRDENAS-MONDRAGÓN G, ULLOA-AGUIRRE A, ISORDIASALAS I, PARRA A, RAMÍREZ-PEREDO J: Anti-prolactin autoantibodies in pregnant women with systemic lupus erythematosus:

- maternal and fetal outcome. *Lupus* 2007; 16: 342-9.
38. GLEZER A: Human macroprolactin biological activity study in Nb2 cells and in Ba/F-03 cells expressing human long prolactin receptor (thesis). São Paulo, São Paulo, University of São Paulo Medical School 2005, 122p.
39. HATTORI N, IKEKUBO K, ISHIHARA T, MORIDERA K, HINO M, KURAHACHI H: Correlation of the antibody titers with serum prolactin levels and their clinical course in patients with anti-prolactin autoantibody. *Eur J Endocrinol* 1994; 130: 438-45.
40. BEN-JONATHAN N, LA PENSEE CR, LA PENSEE EW: What can we learn from rodents about prolactin in humans? *Endocr Rev* 2008; 29: 1-41.
41. SOARES CR, UEDA EK, OLIVEIRA TL, GOMIDE FI, HELLER SR, BARTOLINI P: Distinct human prolactin (hPRL) and growth hormone (hGH) behavior under bacteriophage lambda PL promoter control: temperature plays a major role in protein yields. *J Biotechnol* 2008; 133: 27-35.
42. HANDELSMAN DJ, MCDOWELL IF, CATERSON ID, TILLER DJ, HALL BM, TURTLE JR: Testicular function after renal transplantation: comparison of Cyclosporin A with azathioprine and prednisone combination regimes. *Clin Nephrol* 1984; 22: 144-8.
43. GRZESZCZAK W, KOKOT F, WIECEK A, ZUKOWSKA-SZCZECOWSKA E: Prolactin secretion in kidney transplant patients. *Int Urol Nephrol* 1990; 22: 567-71.
44. KAJDANIUK D, MAREK B, KOS-KUDLA B: Influence of adjuvant chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil on plasma melatonin and chosen hormones in breast cancer premenopausal patients. *J Clin Pharm Ther* 2001; 26: 297-301.
45. KAJDANIUK D, MAREK B: Influence of adjuvant chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil on plasma insulin-like growth factor-I and chosen hormones in breast cancer pre-menopausal patients. *J Clin Pharm Ther* 2000; 25: 67-72.
46. GROSVENOR CE: The release of prolactin in the lactating rat: effect of chloroquine. *Neuroendocrinology* 1984; 39: 64-7.
47. WALKER SE: Bromocriptine treatment of systemic lupus erythematosus. *Lupus* 2001; 10: 762-8.
48. MCMURRAY RW, WEIDENSAUL D, ALLEN SH, WALKER SE: Efficacy of bromocriptine in an open label therapeutic trial for systemic lupus erythematosus. *J Rheumatol* 1995; 22: 2084-91.
49. JARALJ, CRUZ-CRUZ P, SAAVEDRAMA *et al.*: Bromocriptine during pregnancy in systemic lupus erythematosus: a pilot clinical trial. *Ann N Y Acad Sci* 2007; 1110: 297-304.
50. BERWAER M, MARTIAL JA, DAVIS JR: Characterization of an upstream promoter directing extrapituitary expression of human prolactin gene. *Mol Endocrinol* 1994; 8: 635-42.