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Short communication

Platelet serotonin in primary Sjögren's syndrome: Level and relation with disease activity

Helena Sarac [a], Jasenka Markeljevic [b], Gordana Mokrovic [c], Viktorija Erdeljic [d], Nada Bozina [e], Lipa Cicin-Sain [c,*]

[a] Department of Neurology, University Hospital Centre Zagreb and Croatian Institute for Brain Research, School of Medicine, University of Zagreb, HR-10000 Zagreb, Croatia
[b] School of Medicine, University of Zagreb and Department of Internal Medicine, University Hospital Sestre milosrdnice, HR-10000 Zagreb, Croatia
[c] Department of Molecular Biology, Rudjer Boskovic Institute, HR-10000 Zagreb, Croatia
[d] Division of Clinical Pharmacology, University Hospital Centre Zagreb, HR-10000 Zagreb, Croatia
[e] Department of Laboratory Diagnostic, University Hospital Centre Zagreb, HR-10000 Zagreb, Croatia

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ABSTRACT

Primary Sjögren's syndrome (pSS) is chronic autoimmune disorder of unknown ethiopathogenesis. In line with the concept of neuroimmunohormonal dysregulation in inflammatory rheumatic diseases, the aim of this study was to investigate platelet serotonin level (PSL) in patients with pSS and its relation with the activity and duration of the disease.
Significantly lower PSL in pSS patients (N = 61) was shown as compared to healthy controls (N = 103). No correlation was found between PSL and the actual disease activity assessed by the recently developed EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI). Results suggest involvement of the serotonin system in the pathogenesis of pSS.

1. Introduction

Primary Sjögren's syndrome (pSS) is an atypical, autoimmune rheumatic disease characterized by lymphocytic infiltration of exocrine glands. In addition to ocular and oral dryness (*sicca* symptoms), the disease can progress to become a systemic, affecting various organs (Jonsson et al., 2005). In about half of patients, gland inflammation is associated with other autoimmune disorders such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc) or vasculitis, in condition called secondary SS. The ethiopathogenesis of pSS is still unknown, as is true for other autoimmune rheumatic disorders. The concept of neuroimmunohormonal dysregulation in which neurotransmitters, cytokines, and hormones play an important role in susceptibility to inflammatory rheumatic disorders exist also in pSS. Studies exploring these associations, particularly in the context of neurotransmitter serotonin (5-hydroxytryptamine, 5HT) are, however, very scarce. Among others, 5HT contribution to the etiopathogenesis of autoimmune rheumatic disorders is indicated by decreased level of serotonin in platelets of patients diagnosed with RA, SLE and SSc, as well as a significant association of magnitude of its reduction with the disease activity (Meyerhoff and Dorsch, 1981; Zeller et al., 1983; Klimiuk et al., 1989).

In this study we explore the possible alteration of platelet serotonin level (PSL) in pSS and its relation to the actual activity of the disease, assessed by the recently developed clinical index, ESSDAI (European League Against Rheumatism Sjögren's syndrome disease activity index). Correlations between PSL and blood markers of immunoinflammation were also obtained.

2. Materials and methods

2.1. Study subjects

The study included 61 patients of both sexes (56 females, 6 males) diagnosed with clinically definite pSS which require the presence of either anti-Ro/SS-A and/or anti-La/SS-B autoantibodies and/or a positive labial gland biopsy (Vitali et al., 2002). Median age of patients was 59.4 years (range: 34–79), median duration of *sicca* symptoms was 8.2 years (range: 1–30) and median years since diagnosis of pSS was established was 5.5 (range: 1–19). Patients were recruited from the University Hospital Centre Zagreb, the University Hospital Sestre Milosrdnice and the Diagnostic Centre Neuron of the Croatian Institute for Brain Research, Zagreb. The community control group was established for this study and consisted of 103 healthy subjects (77 females, 26 males), recruited from the blood donors at the Croatian Institute for Transfusion Medicine, Zagreb, whose healthy status was checked by written questionnaire. Multi-centre research ethics committee approval was obtained prior to starting this study and written informed consent was obtained from all participants.

* Corresponding author at: Laboratory of Neurochemistry and Molecular Neurobiology, Department of Molecular Biology, Rudjer Boskovic Institute, Bijenicka 54, HR-10000 Zagreb, Croatia. Tel.: +385 1 4561 045; fax: +385 1 4561 177.
E-mail address: cicinsai@irb.hr (L. Cicin-Sain).

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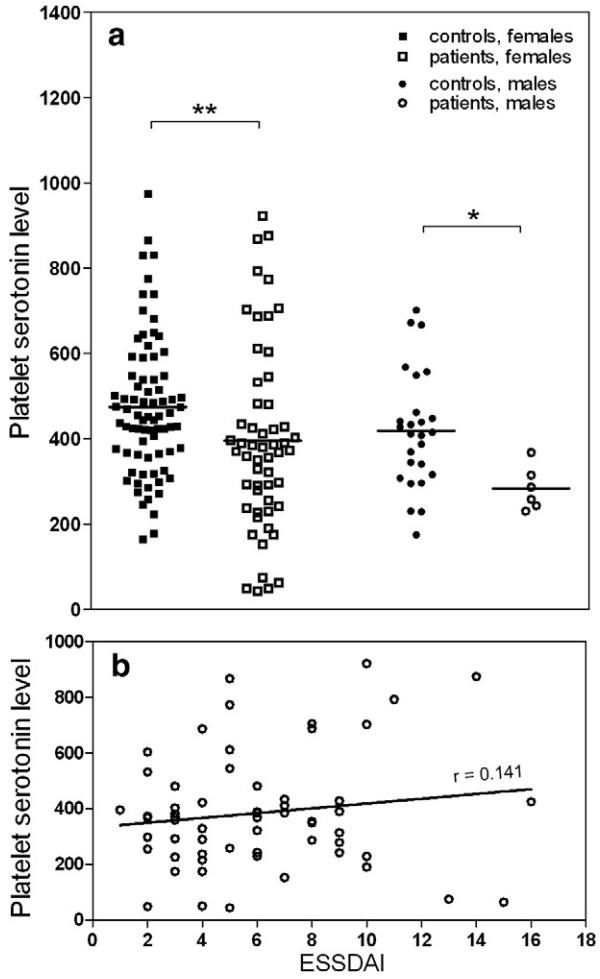

Fig. 1. (a) Individual values of platelet serotonin level, expressed in ng 5HT/10^9 platelets, in male and female patients with primary Sjögren's syndrome and respective healthy controls. Horizontal bars indicate mean values; *$p<0.05$, **$p<0.01$. (b) Correlation of platelet serotonin level and EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) in patients with primary Sjögren's syndrome (N = 61). r = correlation coefficient.

2.2. Clinical assessment

The original diagnostic category was checked up during the study by reported oral and ocular symptoms, Schirmer's test, unstimulated salivary flow, salivary gland biopsy, routine laboratory examination (erythrocyte sedimentation test, C-reactive protein, CRP, haptoglobin, serum proteins, gamma globulins, immunoglobulins, complements C3 and C4), specific biological tests (anti-Ro/SS-A, anti-La/SS-B, antinuclear autoantibodies, ANA, rheumatoid factor, RF) as well as neurological and neuroradiological examination.

All pSS patients received clinical assessment sensitive to the disease activity by using recently published index, ESSDAI (Seror et al., 2010), which consists of 12 organ-specific domains contributing to the disease activity. For each domain, features of disease activity were classified in 3 or 4 levels according to their severity. Data for all possible systemic complications have been taken from all patients with pSS to generate realistic vignettes of systemic involvement.

2.3. Blood sampling and PSL measuring

Venous blood (8 ml) was collected on ACD anticoagulant between 7.30 and 10.00 am. Platelet-rich plasma (PRP) was prepared by centrifugation of blood samples ($200\times g$, 15 min), and aliquoted for platelet counting and PSL determination by orthophthaldialdehyde-enhanced fluorometry, as described previously (Jernej et al., 2000). Blood samples of patients and controls were always processed simultaneously. All measurements were performed in duplicates, with differences between parallels amounting to approximately 10%. Results are expressed as ng 5HT/10^9 platelets.

2.4. Statistical analysis

Results are shown as individual values, means ± standard deviation (SD) or medians with range of values. Differences between pSS patients and healthy controls were evaluated by Student's *t*-test (normally distributed parameters) or Mann–Whitney *U*-test (parameters not normally distributed). The normality assumption was examined by Shapiro–Wilk test. The relationship between PSL and other parameters was evaluated by Pearson correlation coefficient (r), multiple regression analysis and binary logistic regression. Statistical analyses were performed using GraphPad Prism, version 5.02 (GraphPad Software, San Diego, CA, www.graphpad.com) and AnalystSoft, StatPlus, version 2007 (http://www.analystsoft.com). The level of statistical significance was set to 0.05.

3. Results

Individual and group values of PSL measured in patients with pSS and healthy controls are shown in Fig. 1a and Table 1, respectively. A clear difference between patients and controls was found in both sexes, with PSL values being significantly lower in patient groups (all: 385 vs 460 ng 5HT/10^9 platelets). Number and volume of platelets in PRP samples were similar in all groups. Procedure for PRP preparation has been previously validated as yielding approx. 70% of platelets from the whole blood that well represent the entire platelet population (Balija et al., 2011). ESSDAI score of pSS patients ranged from 1 to 16 (median = 6). Correlation analysis of PSL and ESSDAI score showed no association (r = 0.141) of this platelet parameter with disease activity (Fig. 1b). Likewise, reduction in PSL has not been associated with duration of the *sicca* symptoms and years since diagnosis of pSS was established. No correlations of PSL with any of measured circulating serum antibodies or immune complexes were observed either. Data on the presence of antibodies and other blood tests in pSS patients are given in Table 2.

Table 1
Platelet parameters in patients with Sjögren's syndrome and in healthy controls subdivided according gender.

		N	Platelet number $\times 10^9$L/ml PRP	Platelet volume fL	Platelet serotonin level ng 5HT/10^9 platelets	p-Value
Patients	Females	55	316 ± 104	9.57 ± 1.06	396 ± 217	0.0038
	Males	6	307 ± 70	8.87 ± 0.77	283 ± 51	0.0129
	All subjects	61	315 ± 101	9.50 ± 1.05	385 ± 209	0.0009
Controls	Females	77	345 ± 103	9.85 ± 0.98	474 ± 162	
	Males	26	351 ± 92	9.44 ± 0.94	419 ± 131	
	All subjects	103	346 ± 101	9.74 ± 0.99	460 ± 157	

Mean ± SD; N = number of subjects; PRP = platelet-rich-plasma sample; and p-value: patients vs respective control.

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Table 2
Soluble markers of inflammation and autoantibodies in patients with primary Sjögren's syndrome.

Blood parameter	
Positive anti-Ro/SSA antibodies, N (%)	23 (37.7)
Positive anti-La/SSB antibodies, N (%)	16 (26.5)
Antinuclear antibodies (ANA), N (%)	36 (59.0)
Positive rheumatoid factor (RF), N (%)	45 (73.7)
erythrocyte sedimentation rate; mm, median (range)	20 (6–90)
C-reactive protein (CRP), mg/L, mean (SD)	2.30 (0.2–15)
Complement C3, g/l, mean (SD)	1.11 (0.25)
Complement C4, g/l, mean (SD)	0.26 (0.08)
Haptoglobin (hpt), g/L, median (range)	1.43 (0.5–2.9)
Serum albumins, g/L, mean (SD)	46.1 (4.83)
Serum gamma globulins, mean (SD)	12.2 (4.07)
IgG, g/L, median (range)	10.3 (3.9–27)
IgA, g/L, median (range)	2.25 (0.94–4.66)
IgM, g/L, median (range)	1.11 (0.28–4.72)
IgE, g/L, median (range), N = 13	31.2 (3.6–666)

N = number of subjects.

4. Discussion

The role of serotonin in mediating interactions among the immune, nervous and endocrine systems in susceptibility/resistance to inflammatory disorders is emerging (Eskandari et al., 2003; Franco et al., 2007; Ahern, 2011) but studies addressing the relationship between serotonin and autoimmune inflammatory disorders are scarce. In the present study we showed decreased PSL in a large group of clinically well-characterized pSS patients. To our knowledge, this is the first report on alteration of 5HT system in pSS.

Considering both, our results and available literature reports, it seems that reduced PSL is a general finding in autoimmune rheumatic diseases. This could reflect increased 5HT release during platelet activation due to immunoglobulins/immune complexes binding to the platelet surface (Gresele, 1991) or by factors liberated from leukocytes (Totani and Evangelista, 2010). Higher plasma levels of platelet-derived microparticles, which are thought to reflect platelet activation, were shown in pSS as well as in RA and SLE (Sellam et al., 2009). On the other hand, direct measuring of platelet release reaction in SLE patients, showed no correlation with magnitude of PSL reduction (Meyerhoff and Dorsch, 1981), suggesting that increased release of platelet granules constituents cannot provide the sole explanation for decreased PSL.

PSL depletion may also be a consequence of the induction of kynurenine-pathway-enzymes by increased production of proinflammatory cytokines, which then increases/redirects tryptophan catabolism and may affect serotonin biosynthesis (Davies et al., 2010). As platelets accumulate 5HT from the surrounding plasma, their amine content could mirror disturbances in peripheral 5HT synthesis/homeostasis, so the observed PSL reduction may reflect overall hypofunctioning of 5HT system in pSS. In line with this pSS patients often exhibit neuropsychiatric disturbances such as anxiety, depression and stress-like symptoms including fatigue (Johnson et al., 2006), which are thought to be related to hypoactivity of brain 5HT system. Additional mechanisms, such as impaired 5HT transport, may probably contribute to PSL depletion, and studies investigating such a possibility are currently underway in our laboratories.

Absence of correlation of PSL values and disease activity in pSS patients, as contrasted to RA, SLE and SSc, may indicate distinct features of pSS in comparison to other systemic autoimmune disorder, as was highlighted in previous biochemical (Sellam et al., 2009) and genetic studies (Emamian et al., 2006). These results may also be interpreted as patients with pSS often demonstrate a systemic inflammatory reaction of low-grade intensity in contrast to patients with RA and/or SLE, as previously suggested (Valtýsdótir et al., 2001), or they might indicate that course of the pSS disease vary over time and cumulative ESSDAI score would be more applicable for describing disease severity.

In conclusion, decreased PSL in clinically well-defined pSS patients is in line with hypothesis of interrelation between chronic immunoinflammation and 5HT system dysregulation. Large prospective studies are needed to investigate pathological role of 5HT alteration in pSS multisystem dysfunction.

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