Increase in peripheral benzodiazepine receptors on monocytes in fibromyalgia

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Objective. The aim of this study is to evaluate the expression of Peripheral Benzodiazepine Receptors (PBRs) on leukocytes in patients affected by primary fibromyalgia and to argue their possible role in pain perception and in modulation of immunologic process.

Methods. The expression of PBRs has been evaluated by flow cytometry on monocytes, on lymphocytes and on granulocytes in twenty patients with primary fibromyalgia, with indirect immunofluorescence methods.

Results. Upregulation of leukocyte PBRs expression has been demonstrated in fibromyalgia. A statistically significant difference has been documented only in monocytes. The monocyte PBRs expression was 26.74 ± 14.84 MIF in fibromyalgia versus 17.45 ± 8.54 MIF in controls (P < 0.023). Upregulation of PBRs expression, although not statistically significant, was also observed in lymphocytes and granulocytes.

Conclusions. The monocyte PBRs overincrease in fibromyalgia may be due to abnormalities in the regulation of pain or to inflammation. It might perhaps explicate the possible mechanisms of therapeutic response to benzodiazepine in fibromyalgia.

KEY WORDS: Fibromyalgia, Peripheral benzodiazepine receptors, Monocytes, Pain, Inflammation.

Peripheral benzodiazepine receptors (PBRs) are involved in the regulation of pain perception, the modulation of immune and inflammatory systems and the protection of cells from damage caused by oxygen free radicals [1]. Fibromyalgia is not a well characterized rheumatic condition, and patients present with multifurict symptoms and a normal biochemical pattern. Hyperalgesia is often associated with headache, irritable bowel syndrome, panic attacks, myalgias, insomnia, anxiety and depression. Patients exhibit a lower threshold for peripheral nerve ending activation, leading to abnormal pain perception and hyperalgesia. Abnormalities in the release of neurotransmitters (endorphins, benzodiazepine and serotonin), endogenous opioids, inflammatory and anti-inflammatory cytokines, together with abnormal contractility of striated and smooth muscle cells, are described in patients with fibromyalgia [2].

Patients and methods

Twenty patients with primary fibromyalgia, according to the American College of Rheumatology (ACR) criteria, were studied (19 females, one male), aged 32–68 yr (mean ± s.d. 50.1 ± 10.3 yr). Written consent was obtained according to the Declaration of Helsinki. Patients with cancer, infection, psychosis or inflammatory diseases were excluded. Nine patients were housewives, six were managers and five were manual workers. Four patients complained of deep muscle pain, five patients of hyperalgesia to pinprick, five patients of static mechano-allodynia, five patients of non-specific unlocalized muscular pain, eight patients of headache and eight patients of irritable bowel syndrome. No patient was under pharmacological treatment. Controls consisted of 20 volunteer blood donors matched for age and sex. The study was approved by the local ethics committee (Comitato Etico Ospedale Civile di Legnano). Each patient underwent a screening blood test, chest X-ray, ultrasound abdomen examination, total body scintiscan and bone densitometry. PBR expression on leucocyte plasma membranes was evaluated by cytometry with indirect immunofluorescence. The first antibody was anti-PBR (peripheral-type benzodiazepine receptor; Sanofi, Milan, Italy) obtained in chickens by immunization with a synthetic peptide. For indirect immunofluorescence, a fluorescein isothiocyanate (FITC)-labelled anti-chicken monoclonal antibody was used (monoclonal anti-chicken light chain FITC conjugate clone CH 31; Sigma). The latter antibody was also used without anti-PBR to perform a negative control. Acquisition and analysis were performed with a FACScalibur flow cytometer and Cellquest software (Becton Dickinson). The CD45/SSC gating method was used to identify the white blood cell (WBC) subset of lymphocytes, monocytes and granulocytes. The immunofluorescence intensity of each WBC subset was evaluated as mean of positive channel/mean of negative channel. Results were expressed as mean ± s.e. and Student’s t-test for paired samples (SPSS for Windows) was used.

Results

There was greater expression of PBR in fibromyalgia patients than in controls. A statistically significant difference was observed in monocytes (Fig. 1).

Discussion

Higher PBR expression seems to correlate with inflammatory indexes, suggesting that PBRs have a role during inflammatory process [1]. Studies on animal models of inflammation have shown anti-inflammatory, anti-oedema and pain-relieving properties of PBRs [3–5]. In vivo, therapy with ligands of PBRs (PK 11195 and...
Ro5-4864) significantly decrease the release of interleukin (IL)-6 and IL-13 in pleural exudates, thus inhibiting inflammation [6]. Significantly lower expression of PBRs on human mononuclear cells is observed in patients with osteoarthritis than in patients with rheumatoid or psoriatic arthritis [7].

PBRs are also involved in the modulation of immune processes. PBR agonists (Ro 5-4864, PK11195 and diazepam) have been shown to induce strong chemotaxis in monocytes [8]. A high level of PBRs correlates with increased production of anti-inflammatory molecules and with decreased expression of CD11b/CD18, which is a marker of phagocyte activation [9]. PBRs also play a role in the regulation of stress responses and in the modulation of chronic pain due to interaction with the opioid system. Chronic pain syndromes usually associate with increased release of endogenous substances acting as diazepam binding inhibitors (DBIs). DBIs have high affinity for PBRs. Increased expression of PBRs correlates with excessive DBIs and increased production of inflammatory cytokines [8, 9]. In patients with myocardial angina, inflammatory cytokines correlate with lower PBR expression, possibly leading to inflammation-induced pain [8]; PBR expression might correlate with DBIs. Ferrarese et al. observed that chronically anxious patients are characterized by a lower density of PBRs and that long-term treatment with diazepam restores PBR expression to normal values; they argue that DBI release may act as an anxiety substance [10]. In addition, it should be borne in mind that pain perception depends on individual and psychological factors and that the relationships between inflammation and fibromyalgia are not completely explained. In our study we observed that PBRs are increased in fibromyalgia, possibly due to abnormalities in the regulation of the nociceptive system. These data might indicate the mechanisms of the therapeutic response to benzodiazepine drugs in fibromyalgia, although further work is necessary.

Study limitations

Our data are preliminary. The assay of PBRs was performed at baseline and after 3 months in a small number of patients. We did not test DBIs or endorphins or other endogenous ligands for PBRs, which might modulate pain perception and inflammation. The authors have declared no conflicts of interest.

References