Brain, Behavior, and Immunity 53 (2016) 49–53

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Short Communication

Evaluation of periodontitis in hospital outpatients with major depressive disorder. A focus on gingival and circulating cytokines

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ARTICLE INFO

Article history:
Received 24 August 2015
Received in revised form 20 November 2015
Accepted 21 November 2015
Available online 23 November 2015

Keywords:
Interferon-γ
Interleukin-1β
Interleukin-6
Major depressive disorder
Periodontal disease

ABSTRACT

An imbalance in stimulated cytokine production is associated with the etiopathogenesis of numerous diseases such as major depressive disorder (MDD) and periodontal disease. Increased cytokine levels have been reported in the gingival crevicular fluid (GCF) of patients with MDD. Thirty-six outpatients with MDD participated in this study. Each outpatient was age-matched (±3 years) with a healthy control ($n = 36$). The patients were controlled for race and smoking habits. Unstimulated and stimulated interleukin 6 (IL-6), interleukin 1β (IL-1β), and interferon-γ (INF-γ) production in whole blood culture (WBC) and IL-6 and IL-1β levels in the GCF were evaluated. Circulating levels of IL-6 and IL-1β (unstimulated) as well as GCF IL-1β were modestly lower in MDD patients, compared to the levels in age-matched controls (Mann–Whitney, $p = 0.002$, 0.0075, ANCOVA, $p = 0.025$, respectively). In the unstimulated group, there was no correlation between the levels of circulating IL-6 and GCF IL-6 ($r = 0.07$, $p = 0.67$), and between the levels of circulating IL-1β and the IL-1β level in the CGF ($r = -0.08$, $p = 0.63$). In the LPS stimulation group, there was no correlation between the levels of circulating levels of IL-6 and GCF IL-6 ($r = 0.02$, $p = 0.91$) or between the circulating IL-1β and GCF IL-1β ($r = 0.13$, $p = 0.42$). We observed modest immunosuppression in MDD patients (evaluated by no stimulation whole blood culture [WBC]), especially in patients with melancholic depression, chronic depression, and severe depression.

1. Introduction

An imbalance in cytokine production has been associated with the etiopathogenesis of numerous diseases such as major depressive disorder (MDD) American Psychiatric Association, 1994; Miller et al., 2009 and periodontal disease (Gemmell and Seymour, 2004). Periodontal disease is caused by a host reaction to microbial aggression. It is characterized by inflammation of the periodontal tissues in association with increased levels of local proinflammatory cytokines (Page and Kornman, 1997; Orozco et al., 2006). Major depression is a syndrome triggered by biological, social, or psychological factors (e.g., neurotransmitter deficiency, divorce, financial problems, stressful events, low self-esteem) (American Psychiatric Association, 1994). Clinical presentation may include sadness, fatigue, weight change, sleep disturbance and occupational impairment (American Psychiatric Association, 1994). The most accepted theory of depression, the monoamine hypothesis, on which antidepressants are usually

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http://dx.doi.org/10.1016/j.bbi.2015.11.014
0889-1591/

based, explains depression as a change in the activity of monoamine neurotransmitters, particularly serotonin and noradrenalin (American Psychiatric Association, 1994). Unfortunately, some patients fail to respond to conventional medication treatment and some authors speculate that higher inflammatory biomarkers may also contribute to treatment resistance (Miller et al., 2009). Furthermore, inflammation may decrease the neurotransmitter metabolism and neurogenesis (Miller et al., 2009).

Inflammatory markers in the gingival crevicular fluid (GCF) have been analyzed to elucidate the etiopathogenic mechanism of periodontal disease (Orozco et al., 2006). Proinflammatory cytokines such as interleukin-6 (IL-6) and interleukin-1β (IL-1β) are of particular interest because they are associated with alveolar bone resorption, and have been detected in the gingival fluid and in the biopsies of inflamed periodontal tissues (Orozco et al., 2006; Johannsen et al., 2006; Fitzsimmons et al., 2010).

Some authors have demonstrated that stress and depression are associated with higher levels of these mediators at the sulcular level and that these factors could predispose a person to a higher risk of periodontal disease. Increased levels of cytokine in the GCF have been reported in patients with MDD (Johannsen et al., 2006). However, the relationship between cytokine levels in the peripheral circulation and in the GCF has not yet been described. The purpose of our study was to evaluate IL-6, IL-1β and INF-γ levels in whole blood culture (WBC) and IL-6, IL-1β in the GCF in a group of patients with MDD who were otherwise medically healthy. Our goal was to determine the following: (1) IL-6, IL-1β, INF-γ levels in WBC; and IL-6, IL-1β levels in the GCF and (2) (Miller et al., 2009) the correlation between IL-6 and IL-1β levels in WBC and in the GCF. We hypothesized that patients with MDD who express increased levels of circulating cytokines would also have increased levels of cytokines in the GCF, which could explain the relationship between periodontal disease and MDD.

2. Methods and materials

Participants, clinical evaluation and procedure were described were described previously (Solis et al., 2014). Participants' health status was determined by standard clinical evaluation, physical examination and laboratory measurements (TSH, liver function tests, serum creatinine, blood urea nitrogen and complete blood count). All participants were evaluated by one experienced clinical researcher (psychiatrist) trained in the application of all instruments. Exclusion criteria for participants included: use of glucocorticoids topically and/or orally in the last 2 weeks; current use of non-steroidal anti-inflammatory drugs; current episode of acute infection or allergy; current diagnosis of any neurodegenerative or autoimmune disorder; any severe clinical condition; alcohol or drug abuse; acute psychotic symptoms; risk of suicide; any psychiatric comorbidity that could be negatively affected by the use of antidepressant treatment; and any condition that could impair the understanding of the protocol and/or interpretation of the results (e.g., a history of head trauma with post-traumatic amnesia).

2.1. GCF sampling

A Periopaper strip (Proflow Incorporated, Amityville, NY, USA) was inserted into each site until resistance was felt. The paper strip was inserted into each healthy sample site for about 30 s and into inflamed sample sites (gingivitis or periodontitis) for a shorter period of time. GCF volume was quantified with a Periotron 8000 apparatus (Oraflow Inc., Plainview, NY, USA). The plot of the Periotron 8000 units against fluid volume was similar to those found in other studies. Each strip was placed in a cryotube, frozen, and stored at −70 °C until use. The paper strips were re-suspended in 800 μl phosphate buffered saline (PBS), and used for cytokine determination. The eluted IL-1β and IL-6 were multiplied by 0.8 to determine the amount in picograms in each sample.

2.2. Determination of cytokines

Levels of IL-1β, IL-6, and INF-γ were measured in WBCs before and after lipopolysaccharide stimulation (LPS; 1 μg/ml). These methods were also described previously (Marques-Deak et al., 2007).

2.3. Statistical analysis

Periodontal status of the GCF sites was compared by the chi-square test. The Mann–Whitney test and Kruskal–Wallis test were used to compare the groups. Analysis of covariance (ANCOVA) was used to control the cytokine levels for periodontal status. Spearman's correlation coefficient (listwise) was used to evaluate the correlation of the cytokine variables. General linear models (GLMs) were constructed to explain the variability of the systemic cytokines and GCF cytokines. The Minitab (Version 16.2.2), R (Version 2.15.0) and SPSS (Version 23) statistical software were used. Differences at the 5% level were considered significant.

3. Results

3.1. Clinical characteristics of the sample

Healthy periodontal sites sampled were observed in 20 patients without depression (76.92%) and in 19 patients with depression (65.52%). There was no significant difference between the groups, based on the chi square test ($p = 0.352$).

3.2. Comparison of cytokine production

Fifty-five gingival fluid samples were collected. Seventeen samples for IL-6 determination and 32 samples for IL-1β determination were discarded because of inappropriate readings (due to contamination and/or cross-reactivity for example). Tables 1 and 2 shows the comparison between the groups.

3.3. Multivariate analysis

In the GLM models, GCF cytokines levels were not associated with the covariates (i.e., periodontal disease, depression, smoking, and body mass index). Conversely, the levels of unstimulated IL-6 and IL-β were negatively associated with depression (Supplementary material).

3.4. Correlation between the variables studied

In the unstimulated assay, there was a significant correlation between circulating levels of IL-1β and IL-6 ($r = 0.88$, $p < 0.001$), IL-1β and INF-γ ($r = 0.69$, $p < 0.001$), and IL-6 and INF-γ ($r = 0.59$, $p < 0.001$). In the LPS-stimulated group, there was a significant correlation between circulating levels of IL-1β and IL-6 ($r = 0.56$, $p < 0.001$), IL-1β and INF-γ ($r = 0.60$, $p < 0.001$), and IL-6 and INF-γ ($r = 0.39$, $p = 0.01$). In the unstimulated group, circulating levels of IL-6 and GCF levels of IL-6 were not significant correlated ($r = 0.07$, $p = 0.67$). In the unstimulated group, there was also no correlation between circulating levels of IL-1β and GCF levels of IL-1β ($r = -0.08$, $p = 0.63$). In the LPS-stimulated group, there was no correlation between circulating levels of IL-6 and GCF levels