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An increase in salivary interleukin-6 level following acute psychosocial stress and its biological correlates in healthy young adults

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ABSTRACT

Although interleukin-6 (IL-6) has been investigated frequently in stress research, knowledge regarding the biological processes of IL-6 in association with psychosocial stress remains incomplete. This study focused on salivary IL-6 and reports its temporal variation and biological correlates following acute psychosocial stress. Fifty healthy young adults (39 male and 11 female students) were subjected to the psychosocial stress test 'Trier Social Stress Test' (TSST), wherein the participants were asked to deliver a speech and perform a mental arithmetic task in front of 2 audiences. Collection of saliva samples, measurement of heart rate, and assessment of negative moods by visual analogue scales were conducted before, during, and after TSST. Salivary IL-6 levels increased by approximately 50% in response to the TSST and remained elevated for 20 min after the stress tasks were completed. Cluster analyses revealed that individuals with sustained elevation of IL-6 levels following the TSST exhibited a lower cortisol response compared to individuals with lower IL-6 levels. In the correlation analyses, a greater IL-6 response was associated with a higher heart rate during the mental arithmetic task ($r = .351$, $p < .05$) and with a lower cortisol response ($r = -.302$, $p < .05$). This study demonstrates that salivary IL-6 levels are elevated for a relatively long period following acute psychosocial stress, and suggests that sympathetic activity and cortisol secretion are involved in elevation of salivary IL-6 levels.

1. Introduction

Psychosocial stress induces a variety of changes in immune activities. A meta-analysis by Segerstrom and Miller (2004) demonstrated that acute stressors, brief naturalistic stressors (such as exams), and chronic stressors were intricately associated with variations in the number of T-helper lymphocytes, T-cytotoxic lymphocytes, natural killer cells, B lymphocytes, and immunoglobulin. In addition, another meta-analysis indicated that acute stressors induce increases in the levels of circulating inflammatory factors such as interleukin-6 (IL-6), interleukin-1 beta, and C-reactive protein (Steptoe, Hamer, & Chida, 2007). In particular, IL-6 has been frequently investigated with regard to psychosocial stress in recent years (Carroll et al., 2011; Yamakawa et al., 2009). Inflammatory activities have been the focus of these studies because these markers may mediate the influence of psychosocial factors in many diseases.

This study focused on salivary IL-6 levels during and following psychosocial stress because IL-6, in particular, has many functions in the body besides its role in psychosocial stress, an aspect that has been frequently investigated in the literature. In recent years, a number of studies have investigated the cytokine levels in saliva; salivary IL-6, in particular, has been relatively well investigated. IL-6 levels in saliva have been reported not to correlate with those

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in plasma or serum (Fernandez-Botran, Miller, Burns, & Newton, 2011; Minetto et al., 2005, 2007; Sjögren, Leanderson, Kristenson, & Ernerudh, 2006). Increased IL-6 levels in saliva are probably associated with the activity of the salivary glands such as the acinar cells of the salivary glands, oral epithelial cells, periodontal ligament cells, and gingival fibroblasts (e.g. Okada et al., 1997; Sun, Emmert-Buck, & Fox, 1998; Tishler, Yaron, Shirazi, Yossipov, & Yaron, 1999; Yao, Wei, Li, & Hosoi, 2005). Salivary IL-6 may reflect a localised level of inflammation that is relatively independent of the general systemic level of inflammation, and salivary IL-6 could be involved in the mucosal immunity that protects against foreign antigens. Although majority of the studies have investigated salivary IL-6 in patients with diseases of the oral cavity, some studies have also investigated the association between salivary IL-6 and psychosocial stress (Keller, El-Sheikh, Vaughn, & Granger, 2010; Lester, Brown, Aycock, Grubbs, & Johnson, 2010; Sjögren et al., 2006). In 2 such studies, an association between higher salivary IL-6 levels and depression, anxiety, cynicism, and hopelessness was observed in the samples from both middle-aged people and children (Keller et al., 2010; Sjögren et al., 2006). Lester et al. (2010) also reported that salivary IL-6 levels increased in response to prolonged stress (a series of practical examination of gross anatomy) in occupational therapy students.

However, only few studies have reported the relationship between psychosocial factors and salivary IL-6 levels, most of them demonstrating just a correlative relationship between the 2. These studies did not investigate the effects of psychosocial stress on salivary IL-6 in laboratory-controlled settings; moreover, no studies demonstrated either time-series variations of salivary IL-6 levels in response to psychosocial stress or any related biological correlates. Previous studies that investigated circulating IL-6 levels showed that these levels responded to acute psychosocial stress and the magnitude of the response was positively associated with autonomic activities (Owen & Steptoe, 2003; Steptoe, Owen, Kunz-Ebrecht, & Mohamed-Ali, 2002) and was negatively associated with the cortisol response (von Känel, Kudielka, Preckel, Hanebuth, & Fischer, 2006). Therefore, additional research on salivary IL-6 is required to explore the effects of acute psychosocial stress and its biological correlates. Exploring the biological correlates of salivary IL-6 in relation to psychosocial stress could facilitate a better understanding of the biological mechanism involved. Although a previous study showed several possible biological mechanisms of circulating IL-6, these mechanisms have not been elucidated fully (Steptoe et al., 2007).

The purpose of this study was to investigate the variations of salivary IL-6 in response to acute psychosocial stress in healthy young adults. The secondary purpose was to investigate the association between the variation in IL-6 levels and other biological variables (heart rate, parasympathetic activity, and cortisol level). We hypothesised that salivary IL-6 would respond to acute psychosocial stress and that the magnitude of the response would be positively associated with autonomic activities and negatively associated with the cortisol response. Furthermore, to explore the biological correlates, we attempted to categorise the IL-6 response patterns and investigated their associations with autonomic activities and the cortisol response.

2. Methods

2.1. Participants

Thirty-nine male students and 11 female students, from 3 universities located in Tokyo and Saitama Prefectures, participated in this study. They were recruited via advertising in the selected universities. Participants who reported physiological and psychiatric diseases or who used medications or dietary supplements that affect the activity of the hypothalamus–pituitary–adrenal (HPA) or immune system were excluded from the study. Any influence of sex hormones on HPA axis, immune activity, and autonomic activity was minimised by having the female students participate during the late luteal or early follicular phase of their menstrual cycle. Written informed consent was obtained, and the study was approved by the Waseda University ethics committee.

2.2. Measurements

Saliva samples were collected for the measurement of levels of IL-6 and cortisol. The participants were asked to pool saliva in the mouth for a few minutes and to drool their saliva down through a short plastic straw into a collection vial (the passive drool method: for details, see Granger et al., 2007). This collection method was recommended by previous studies because collection methods using cotton, such as Salivette, interfered with the results of salivary immunoassay for IL-6 (Minetto et al., 2007). Saliva samples were stored at −20 °C until the assays were performed.

An electrocardiogram was continuously recorded on a Holter monitor (FM120; Fukuda Denshi, Tokyo), and the heart rate (HR) during the experimental session was computed. As an index of HR variability, the square root of the mean of the sum of the squared successive differences (RMSSD) was also calculated. The RMSSD has been shown to be positively correlated with vagal cardiac control (Berntson et al., 1997; Goedhart, van der Sluis, Houtveen, Willemsen, & de Geus, 2007; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). We used the RMSSD as an indicator of parasympathetic activity.

Negative moods during the experimental session were assessed using 6 Visual Analogue Scales (VAS). Each VAS assessed 6 moods (tension and anxiety, depression, anger and hostility, vitality, fatigue, and confusion) and the participants were asked to respond to the VAS during the experiment. The negative moods score was computed by averaging the scores for the 6 moods (the vitality score was reversed). Oka, Takenaka, and Sakata (1994) reported that the total score of the 6 moods was significantly correlated with Total Mood Disturbance score of the Profile of Moods Scale ($r = .726$) and a state of anxiety ($r = .715$) that was measured by the State-Trait Anxiety Scale. In this study, the VAS was used to assess psychological stressfulness of the assigned tasks.

2.3. Procedure

To minimise the circadian variations of cortisol and IL-6, all experimental sessions started after 2:00 PM and ended before 7:30 PM. We previously confirmed that the levels of salivary IL-6 are relatively stable during this period (Izawa, Miki, Liu, & Ogawa, 2013). In the present study, the participants were instructed to maintain their regular activities, including sleep patterns, the day before the experiment and were asked to refrain from eating, drinking, and strenuous exercise at least 1 h before the experimental session. The participants were subjected to the psychosocial stress test 'Trier Social Stress Test' (TSST), in which the participants were asked to deliver a speech and perform a mental arithmetic task (Kirschbaum, Pirke, & Hellhammer, 1993). On arrival, before they were introduced to the tasks, the participants rested for 10 min (baseline: BL). Following the introduction, they were asked to prepare a public speech for 10 min (preparation period: PR), deliver the speech for 5 min (speech period: SP), and perform a mental arithmetic task for 5 min (mental arithmetic task period: MA). These tasks were performed in front of 2 audiences and the performance was recorded by a video camera and microphone. Thereafter, the subjects rested for 60 min (recovery period: RE).

Collection of saliva samples and assessment of negative moods (VAS assessments) were conducted 9 times: after cessation of each period (BL, PR, SP, and MA), and 10, 20, 30, 45, and 60 min after the start of RE (RE1, RE2, RE3, RE4, and RE5, respectively).

2.4. Salivary assays

The samples were first thawed, and then centrifuged at 3000 rpm for 15 min. The concentration of cortisol in saliva was determined by an enzyme immunoassay using an ELISA Kit (IBL International, Hamburg, Germany). The inter- and intra-assay variations were below 7.3% and 9.3%, respectively. The concentration of IL-6 in saliva was also determined by an ELISA kit (Quantikine High Sensitivity human IL-6 immunoassay; R&D Systems. Abingdon, UK). A 1:4 dilution was chosen for salivary IL-6 measurements, as previously validated (Minetto et al., 2005). The inter- and intra-assay variations were less than 3.5% and 5.0%, respectively.

2.5. Statistical analyses

The mean HR and RMSSD during each period (BL, PR, SP, MA, RE1, RE2, RE3, RE4, and RE5) was computed. Measurement of the cortisol concentration in 1 male participant failed due to an insufficient volume of saliva; therefore, those data were excluded from the analyses. IL-6 concentrations were logarithmically transformed because the Kolmogorov–Smirnov test indicated a skewed distribution of the IL-6 concentrations. The values showed a normal distribution after transformation. In the preliminary analyses, we further calculated salivary flow rate by dividing the sample volume by the time taken to provide the sample and investigated the effects of saliva flow rate on salivary IL-6 concentration. No significant correlations were found between saliva flow rate and IL-6 concentration, and therefore, we did not correct for saliva flow rate in the analyses.