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Preliminary studies of the effects of psychological stress on circulating lymphocytes analyzed by synchrotron radiation based-Fourier transform infrared microspectroscopy

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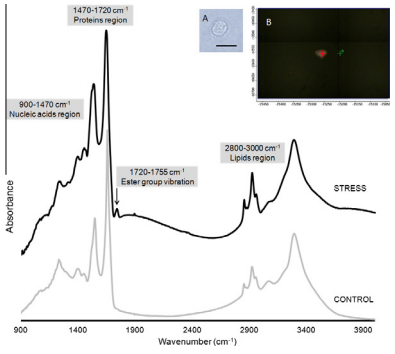
HIGHLIGHTS

- Biochemical changes by psychological stress in lymphocytes investigated by vibrational spectroscopy at single cell level.
- The results showed an increased absorption at lipid peroxidation region in lymphocytes from stressed rats.
- Changes in peak position and absorbance in nucleic acids were observed suggesting reduction of transcriptional activity.
- Results unravel part of the mechanisms by which psychological stress affects immune system with systemic consequences.

GRAPHICAL ABSTRACT



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ABSTRACT

Psychological stress is a condition that not only generates behavioral disorders but also disrupts homeostasis and immune activity that can exacerbate or lead to inflammatory diseases. The aim of this work was to study biochemical changes in circulating immune cells from rats under psychological stress by using vibrational spectroscopy. A stress model was used, where exposure to a stressor was repeated for 5 days. Subsequently, circulating lymphocytes were examined for their biomolecular vibrational fingerprints with synchrotron radiation based-Fourier transform infrared microspectroscopy. The results showed an increased absorption at the ester lipid region (1720–1755 cm^{-1}) in lymphocytes from stressed rats, suggesting lipid peroxidation. Statistical significant changes in wavenumber peak position and absorbance in the nucleic acid region were also observed (915–950 cm^{-1} Z-DNA, 1090–1150 cm^{-1} symmetric stretching of P—O—C, 1200–1260 cm^{-1} asymmetric PO_2 and 1570–1510 cm^{-1} methylated

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ROS
Z-DNA

nucleotides) which suggest a reduction of transcriptional activity in lymphocytes from stressed rat. These results unravel part of the mechanisms by which psychological stress may affect the immune system leading to systemic consequences.

Introduction

Bidirectional relationships exist between the immune and central nervous systems and occur during situations such as physical and psychological stress. These interactions have been shown to be capable of affecting the activities of both lymphocytes and macrophages [1]. Acute or chronic psychological stress can cause pathophysiological changes that disrupt homeostasis and in turn aggravate disease [2,3]. Previous investigations have studied how different kinds of stressors cause the development of a pro-inflammatory response in the brain and in other systems. The process is characterized by events such as the release of cytokines and prostanoids, transcription factor activation and the production of free radicals [4]. It is known that naturalistic stressors in humans can cause immune suppression [5]. Similarly exposure of rats to predator odor (cat) leads to immunosuppression and reduced infection resistance [6] as psychological stress would do in humans [5].

The relationship between stress and immune function has been demonstrated in many contexts including proliferative response to mitogens and cellular activity [7]. For example, psychosocial stress in humans has been related with cancer development by the increased levels of 8-hydroxydeoxyguanosine, a biomarker of oxidative DNA damage, in peripheral blood leukocytes [8]. On the other hand, lymphocyte function is also important to support learning and memory [9] but maladaptation to psychological stress can be severely increased if the T lymphocyte population is deficient [10].

Fourier Transform Infrared (FTIR) spectroscopy is extensively used in investigations of biological samples. Many important biomolecules, such as nucleic acids, proteins, lipids, and carbohydrates, have well-known vibrational fingerprints and can be identified simultaneously without any staining [11]. The use of a microscope allows the localization of these different species within the sample. A common application of this technique is to distinguish healthy and pathological samples, by measuring differences in the quantity and distribution of specific biomolecules [12]. The objective of this work was to use the Synchrotron Radiation Fourier Transform Infrared microspectroscopy (SR-μFTIR) to study the biochemical changes induced by psychological stress in circulating lymphocytes. Here we provide evidence, for the first time at individual cell level, of the effect that psychological stress can have on lymphocytes, one of the safeguarding cells of the body as a consequence of psychoneuroimmunological interactions.

Methods

Animals

Wistar rats were housed with littermates for the first 30 days after birth. Subsequently, two experimental males were transferred into individual cages, under standard conditions at a temperature of 24 ± 2 °C and with a 12 × 12 h dark/light cycle (lights turned on at 8:00 a.m.). Food and water were available to the animals *ad libitum*. At day 60, the juvenile rats with weights of 233 and 235 g were used as the control and psychological stress subjects, respectively. All experimental procedures were approved by the institutional Bioethical Committee in accordance to international standards.

Stress exposure

Adopting the method described by Dielenberg and McGregor [13], the rats were placed into individual avoidance chambers for two periods of time. The first period consisted of 20 min to allow environment habituation in the avoidance chamber, following which the animal was returned to the housing cage for 20 min to relax. Subsequently, the animals were placed a second time for an additional 20 min in the avoidance chamber containing a 25 × 25 cm piece of cotton fabric, either without odor (control) or impregnated with cat odor (stress). The experimental procedure was repeated for five consecutive days between 8:00 and 9:00 a.m. The cotton fabric was impregnated with cat odor by using it as a carpet for a housed adult cat. Each fabric specimen was stored in airtight plastic bags and maintained at −20 °C until used.

Blood sample collection and lymphocyte isolation

On the 6th day of odor treatment the animals were anesthetized using peritoneal pentobarbital. Blood samples were taken using vacutainer™ EDTA tubes, by cardiac puncture and approximately 4 mL were obtained per rat. Whole blood was diluted with one volume of PBS-EDTA at pH 7.4 (137 mM NaCl; 8.2 mM Na_2HPO_4; 1.5 mM KH_2PO_4; 3.2 mM KCl and 4 mM EDTA). Subsequently 4 mL of this dilution was placed onto 4 mL of a isotonic Percoll solution bed (40% PBS-EDTA, 57.3% Percoll™ and 2.7% 10X PBS) and centrifuged at 1000*g* for 10 min. Lymphocytes appearing in the gradient interface were separated and washed twice by homogenizing with 12 mL PBS-EDTA and re-centrifuged [14]. The final cell pellet was suspended in 1.5 mL of PBS-EDTA and aliquots of 300 μL were then transferred into a microtube containing 4% paraformaldehyde for 20 min at room temperature in order to fix the cells. The fixed cells were precipitated with a 30 s pulse then washed in 1 mL of deionized water and centrifuged for 30 s following which they were resuspended in 200 μL deionized water. Fifteen microlitre of the cell suspension were smeared onto a microscope slide for reflective IR measurements and gently heated to evaporate the liquid. Samples were transported at room temperature to beamline ID21 at European Synchrotron Radiation Facility (ESRF) to be analyzed by SR-μFTIR. Samples were kept inside a desiccator at room temperature until measurements were performed.

White blood cell type determination

In order to confirm which type of white blood cells were isolated [14], four different rat blood lymphocytes isolations were characterized and counted using a Sysmex XT-1800i Hematology Analyzer.

SR-μFTIR

The SR-μFTIR measurements were performed on single cells prepared as previously described, using the FTIR spectromicroscopy end-station of ID21 at the ESRF. The single cell spectra were collected in reflection mode using an IR microscope (Thermo Nicolet Continμum) coupled to a FTIR spectrometer (Thermo Nicolet Nexus). The IR microscope is equipped with a 32× objective, a motorized sample stage and a liquid nitrogen cooled 50 μm mercury cadmium telluride detector. In order to match the beam