Sleep Science

ORIGINAL ARTICLE

Chitotriosidase as a marker of macrophage activation after paradoxical sleep deprivation

Quitotriosidase como um marcador de ativação de macrófagos após a privação de sono paradoxal

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ABSTRACT

Objectives: Chitotriosidase (CHIT) is a functional chitinase secreted by activated macrophages. Considering that sleep is an important physiological event that directly influences health and is related to immune system function, our aim is to verify whether the activity of CHIT is altered after paradoxical sleep deprivation (PSD) and to assess the extent to which recovery sleep could affect this biological process. **Methods:** We analyzed CHIT enzymatic activity of Swiss and C57BL/6 mice and Wistar rats submitted to PSD for 72 hours without sleep rebound and with 24 hours of free sleep following PSD. **Results:** We observed an increase in the activity of plasma CHIT in PSD Swiss mice when compared to the Control group (CT) ($p < 0.05$), C57BL/6 mice ($p < 0.05$) and Wistar rats ($p < 0.05$). After 24 hours of sleep, no differences were observed in rebound group (RG) compared to PSD Swiss mice ($p > 0.05$). Moreover, differences were significant among RG C57BL/6 mice ($p < 0.05$) and RG Wistar rats ($p < 0.01$) compared with their respective PSD groups. **Conclusions:** Our data suggest that CHIT is a relevant marker of macrophage activation, not only for infections and diseases but also after 72h of PSD in mice and rats.

Keywords: immune system, mice, rats, sleep deprivation.

RESUMO

Objetivos: Quitotriosidase (CHIT) é uma quitinase funcional secretada por macrófagos ativados. Considerando-se que o sono é um importante evento fisiológico que influencia diretamente a saúde e está relacionado com a função do sistema imunológico, o nosso objetivo é verificar se a atividade de CHIT é alterada após a privação de sono paradoxal (PSP) e avaliar a extensão em que a recuperação do sono pode afetar este processo biológico. **Métodos:** Nós analisamos a atividade enzimática da CHIT de camundongos suíços e C57BL/6 e de ratos Wistar submetidos à PSP por 72 horas e com 24 horas de rebote de sono após a PSD. **Resultados:** Observamos um aumento na atividade de CHIT no plasma de camundongos após PSP quando comparado com o grupo controle (CT) ($p < 0,05$), C57BL/6 ($p < 0,05$) e ratos Wistar ($p < 0,05$). Quando os animais tiveram 24 h de rebote de sono, não houve diferença entre os camundongos suíços ($p > 0,05$) comparados com os submetidos à PSP, mas diferenças foram significativas entre os camundongos C57BL/6 ($p < 0,05$) e ratos Wistar ($p < 0.01$) em comparação com o seus respectivos grupos PSP. **Conclusões:** Nossos dados sugerem que CHIT é um marcador de ativação de macrófagos relevante, não só para infecções e doenças, mas também após 72h de PSP em camundongos e ratos.

Descritores: camundongos, privação do sono, quitinase, ratos, sistema imunológico.

INTRODUCTION

Chitotriosidase (CHIT) is a functional chitinase secreted by activated macrophages. Its enzymatic activity is markedly elevated in plasma of patients suffering from lysosomal lipid storage disorders, sarcoidosis, thalassemia, and visceral Leishmaniosis[1-3]. CHIT is selectively expressed in chronically activated tissue macrophages such as the lipid-laden storage cells that accumulate in large quantities in various tissues of Gaucher Disease patients[4]. Tissue macrophages largely secrete newly synthesized 50-kDa CHIT, but about one-third is directly routed to lysosomes and proteolytically processed to a 39-kDa unit that remains catalytically active[5]. Intriguingly, individuals from various ethnic groups carry one abnormal CHIT gene with a 24-bp duplication that prevents production of enzyme[6].

Therefore, approximately 6% of the population is homozygous for this mutant allele and consequently lacks CHIT activity[7]. Analogous to the function of homologous chitinases in plants, the physiological role of CHIT is most likely involved in innate immunity toward chitin containing pathogens. Moreover, an increased risk for nematode infection has indeed been described for CHIT-deficient individuals[7]. Recently, was observed that prolactin, which is structurally related to several cytokines and is involved in regulating monocyte/macrophage functions, upregulates CHIT gene expression in human macrophages, suggesting that CHIT is not only a biochemical marker of macrophage activation in lysosomal diseases and hematological disorders, but also may reflect challenges to immune system[8].

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Sleep deprivation is an increasingly common condition in modern life and results in predisposition to many disorders. Sleep loss has been associated with increased cardiovascular risk[9], psychological stress, and immune system impairment[10,11]. Ultimately, short sleep time has been associated with high risk of mortality in humans[12-14]. Interestingly, some studies have shown that immunization efficiency is reduced during sleep deprivation or restriction[10,14,15]. Because sleep deprivation is an emerging research field, the understanding of the physiological aspects of this condition is far from complete. Therefore, our aim is to verify whether the activity of CHIT is altered after PSD and to assess the extent to which recovery sleep could affect this biological process.

MATERIALS AND METHODS

Animals

Male Swiss and C57BL/6 mice and Wistar rats (3 months of age) from the colony maintained by the Department of Psychobiology - Universidade Federal de São Paulo (UNIFESP) were used in this study. Animals were maintained on a light-dark 12:12 cycle under controlled temperature conditions (20 ± 2ºC) with free access to food and water. Animals used in this study were maintained and treated in accordance to the guidelines established by the Ethical and Practical Principles of the Use of Laboratory Animals[16] (CEP UNIFESP nº 0183/08). Each type of animal, Wistar rats and Swiss or C57BL/6 mice, consisted of three groups: control (CT), paradoxical sleep-deprived (PSD) and rebound group (RG).

Sleep deprivation

A group for each lineage and species was sleep deprived for 72h by the multiple platform technique for rats[17] or adapted for mice[18] and allowed to sleep for 24h (RG) after sleep deprivation.

Enzymatic activity

Blood was collected by decapitation of animals in tubes containing heparin. After centrifugation for 10 minutes at 3000 *rpm*, plasma was collected and stored at -80ºC until biochemical analysis. Plasma CHIT determinations was based on the method described by Hollak et al.[1]. Briefly, 100 µl of plasma was previously acidified with 10 µl of 0.2 mol/l of citric acid to better achieve the optimum pH for the reaction. A total of 5 µl of the acidified plasma were added to micro-plate. An elution buffer (0.2 mol/l citrate-phosphate; pH 5.2) was added to samples (always on duplicates) and to the blank (acidified albumin solution).

The reaction was incubated for 30 min and it was stopped with 0.3 mol/l glycine/

NaOH buffer (pH 10.6). Enzyme activity is expressed as nmol of hydrolyzed substrate/ml/h. Measurements were performed in a 96-well microplate by a fluorimetric assay using 5 µL of acidified plasma[19]. CHIT enzyme activity is determined by cleavage of the fluorimetric substrate 4-methylumbelliferyl-β-N-N'-N"-triacetylchitotriose, resulting in the release of the fluorescent molecule 4-methylumbelliferone (4-MU), which in alkaline pH emits fluorescence proportional to the amount of molecules hydrolyzed.

Statistical analysis

Comparisons were performed by Kruskal-Wallis analysis followed by a post hoc Multiple Comparisons test. Calculations were performed using Prism™ version 4.03 for Windows (GraphPad, USA). The level of significance was set at $p < 0.05$.

RESULTS

We observed an increase in the activity of plasma CHIT in PSD Swiss mice when compared with the CT group (Figure 1A) ($p < 0.05$, Kruskal-Wallis test followed by *post hoc* multiple comparisons test). The activity returned to normal ranges when the mice had 24h of sleep rebound. After repeating the same experiment in PSD C57BL/6 mice, we also observed increased CHIT activity when compared with the CT and RG ($p < 0.02$; Kruskal-Wallis test followed by post hoc Multiple comparisons test, $p < 0.05$) (Figure 1B). CHIT activity of 72h plasma from PSD Wistar rats also increased when compared with CT and RG ($p < 0.003$, Kruskal-Wallis test followed by post hoc comparisons test, $p < 0.05$ and 0.01, respectively), (Figure 1C).

DISCUSSION

CHIT and other chitinases catabolize chitin, the second most abundant polysaccharide in nature. Chitin is commonly found in lower organisms such as fungi, crustaceans, and insects, but not in mammals. Several studies have mentioned that the CHIT present in mammalian organisms has the function to degrade chitinous structures of invading species[20,21]. Although the biological function of CHIT remains unclear, it is a marker of activated macrophages. Elevated levels of plasma CHIT were also found in disorders caused by abnormal activation of the immune system (e.g.: sarcoidosis[22]) and were associated to the severity of atherosclerotic lesions[23,24]. Plasma CHIT activity is increased in African children with acute *Plasmodium falciparum* malaria and visceral Leishmaniosis[1,25] and neonates with fungal and bacterial infections[26]. These data point to the possible function of inflammatory process modulation by CHIT. Lee et al.[27] also showed in studies *in vitro* that CHIT1 (the chitinase isoform) interacts with TGF-β1 to augment fibroblast TGF-β receptors 1 and 2 expression and TGF-β-induced Smad and MAPK/ERK activation. These studies indicate that CHIT1 is a potential biomarker for interstitial lung disease (ILD) in scleroderma. Di Rosa et al.[8] evaluated by quantitative real-time PCR the mRNA CHIT levels in human monocytes/macrophages following treatment with pro-inflammatory stimuli such as interferon-gamma (INFγ), tumor necrosis factor-alpha (TNFα), lipopolysaccharide (LPS), and interleukin-10 (IL-10), an anti-inflammatory cytokine. Stimulation of macrophages with INFγ, TNFα and LPS resulted in increased levels of CHIT mRNA, as well as CHIT activity, whereas IL-10 decreased CHIT synthesis.

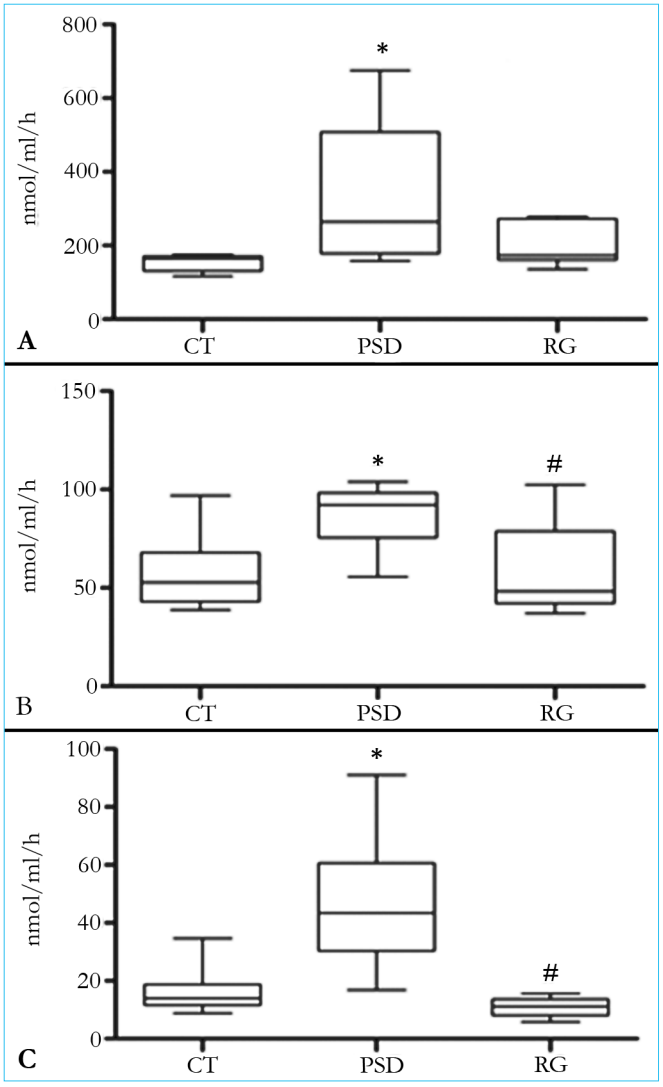

Figure 1. A: Plasma CHIT activity of Swiss mice. There was significant difference between groups (Kruskal -Wallis $p < 0.05$) post hoc multiple comparisons test (PSD * $p < 0.05$ vs. CT); B: Plasma CHIT activity of B157/6 mice. There was significant difference between groups (Kruskal-Wallis $p < 0.02$) post hoc multiple comparisons test (PSD vs. CT * $p < 0.05$; PSD vs. RG $^{\#}$ $p < 0.05$); C: Plasma CHIT activity of Wislar rats. There was significant difference between groups (Kruskal-Walis $p < 0.003$) post hoc mtltiple comparisons test (PSD vs. CT * $p < 0.05$; PSD vs. RG $^{\#}p < 0.01$). PSD: paradoxical sleep deprivation; RG: rebound group; CT: control.

Malaguarnera et al.[28] observed that prolactin hormone treated monocyte-derived macrophages showed an enhanced superoxide anion (O_2^-) release. Once activated macrophages are also related to the secretion of reactive oxygen species, it is quite possible that there is a relationship between the activity of CHIT and increased levels of oxidative stress as a way of immune system activation.

Sleep deprivation is involved in many physiological and behavioral changes reported in different experimental models. Everson and Toth[14] noted that sleep deprived animals showed that bacterial invasion in body tissues that are normally sterile had a potential role in advanced morbidity, suggesting a host defense impairment. There are also many studies that have reported the involvement of free radical generation during reduction of sleep[29-31]. In a recent study by our group, we found increased activity and mRNA expression of superoxide dismutase in splenocytes of mice deprived of sleep for 72 hours[32]. Here, we observed that CHIT activity in plasma was increased after PSD compared to the CT group (figure 1 A, B and C) in Swiss and C57BL/6 mice and, in Wistar rats. It was also noticed that CHIT activity returned to normal values when these animals were allowed to sleep for 24h. These results provide information about physiological changes induced by PSD and suggest a link between the activation of macrophages and the immune system because sleep plays an important role in the process of body defense[14].

It is interesting to observe that CHIT activity was increased in two different species, rats and mice, as well as in mice of two different lineages, Swiss and C57BL/6. Besides no recognized role for CHIT in superior organisms was described until now this finding point out that it is an enzyme evolutionarily maintained in some mammals as a protective tool against infection by pathogenic agents and natural allergens or ultimately, as a marker of immune system activaction. CHIT is secreted from innate immune cells and main macrophages and its activity is increased in various types of infections such as *Candida albicans* in mice[33], *Cryptococcus neoformans* in humans[21], *Aspergillus fumigates* in Guinea pigs[34], and *Plasmodium falciparum* in humans[3], among others. Because sleep has an important role for the maintenance and efficiency of the immune system[10,11,14,15], our data suggest that CHIT is a relevant marker of macrophage activation, not only for infections and diseases but also after 72h of PSD in mice and rats. This suggests a relationship between sleep and macrophage response and could be considered a marker for insufficient sleep.

DECLARATIONS OF INTEREST

All authors have declared that there is no conflict of interest that could be perceived as prejudicing the impartiality of the present study.

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