

Anti-stress effect of theanine on students during pharmacy practice: Positive correlation among salivary α -amylase activity, trait anxiety and subjective stress

Keiko Unno ^{a,*}, Naoki Tanida ^a, Naoto Ishii ^a, Hiroyuki Yamamoto ^a, Kazuaki Iguchi ^a, Minoru Hoshino ^a, Atsushi Takeda ^a, Hayato Ozawa ^c, Tsutomu Ohkubo ^c, Lekh Raj Juneja ^c, Hiroshi Yamada ^b

^a Laboratory of Bioorganic Chemistry, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

^b Division of Drug Evaluation & Informatics, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

^c Taiyo Kagaku Co., Ltd., Yokkaichi, Japan

ARTICLE INFO

Article history:

Received 23 May 2013

Received in revised form 26 August 2013

Accepted 7 September 2013

Available online 16 September 2013

Keywords:

Theanine

Salivary α -amylase

Anti-stress

Chronic stress

Trait anxiety

Subjective stress

ABSTRACT

Purpose: Theanine, an amino acid in tea, has significant anti-stress effect on experimental animals under psychosocial stress. Anti-stress effect of theanine on humans was evaluated in 5th-year university students during pharmacy practice.

Method: The study design was a single-blind group comparison and participants ($n = 20$) were randomly assigned to theanine or placebo groups. Theanine or placebo (lactose) tablets (200 mg, twice a day, after breakfast and lunch) were taken from 1 week prior to the pharmacy practice and continued for 10 days in the practice period. To assess the anxiety of the participants, the state–trait anxiety inventory test was carried out before the pharmacy practice. Salivary α -amylase activity (sAA) was measured as a marker of sympathetic nervous system activity.

Results: In the placebo-group, sAA in the morning (pre-practice sAA) was higher than in theanine-group during the pharmacy practice ($p = 0.032$). Subjective stress was significantly lower in the theanine-group than in the placebo-group ($p = 0.020$). These results suggest that theanine intake had anti-stress effect on students. Furthermore, students with higher pre-practice sAA showed significantly higher trait anxiety in both groups ($p = 0.015$). Similarly, higher pre-practice sAA was correlated to shorter sleeping time in both groups ($p = 0.41 \times 10^{-3}$).

Conclusion: Stressful condition increased the level of sAA that was essentially affected by individual trait anxiety. The low levels of pre-practice sAA and subjective stress in the theanine-group suggest that theanine intake suppressed initial stress response of students assigned for a long-term commitment of pharmacy practice.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Chronic psychosocial stress is associated with the development of depression, mood disorders, as well as cardiovascular and other age-related diseases (McEwen and Magarinos, 1997; Pedersen et al., 2001; Gareri et al., 2002; Bellinger et al., 2008; Thayer et al., 2010). Intervention of stress-induced alterations with dietary supplements is a potential therapeutic strategy for a healthy life. We have previously

reported that the intake of theanine (γ -glutamylethylamide) suppressed the stress-derived malfunctions in aged mice that were chronically stressed under the confrontational housing (Unno et al., 2011, 2013). Theanine (L-theanine) is the most abundant amino acid in tea. The sweet umami taste of green tea is due to amino acids, especially theanine. Several studies have reported that theanine exerts neuroprotective effects (Nagasawa et al., 2004; Egashira et al., 2004, 2007, 2008; Cho et al., 2008; Kim et al., 2009), modulates the activity of neurotransmitters (Yamada et al., 2007; Kakuda et al., 2008) and reduces psychological stress (Kimura et al., 2007). In this study, we aimed to investigate the effect of theanine supplementation on stress responses in 5th-year college students of the school of pharmaceutical sciences. They were assigned to practice outside the university such as in a hospital or a drug store, for 11 weeks. Such a long-term commitment in new environments provides a stressful condition for young students. Salivary α -amylase activity (sAA), an oral cavity enzyme, was measured as a stress marker (Nater and Rohleder, 2009). Two main body systems

Abbreviations: ANS, autonomic nervous system; HPA, hypothalamus–pituitary–adrenal; sAA, salivary α -amylase activity; pre-practice sAA, sAA in the morning; post-practice sAA, sAA in the evening; STAI, the state–trait anxiety inventory; VAS, visual analog scales.

* Corresponding author at: University of Shizuoka, School of Pharmaceutical Sciences, Laboratory of Bioorganic Chemistry, Yada 52-1, Suruga-ku, Shizuoka 422-8526, Japan. Tel.: +81 54 264 5731; fax: +81 54 264 5909.

E-mail address: unno@u-shizuoka-ken.ac.jp (K. Unno).

are involved in the stress response, the autonomic nervous system (ANS) and the hypothalamus–pituitary–adrenal (HPA) axis. Measurement of sAA has been demonstrated as a useful tool for monitoring ANS reactivity to stress (Nater and Rohleder, 2009). This enzyme is increased rapidly in response to physiological and psychosocial stress (Almela et al., 2011; Nater et al., 2005, 2006; Rohleder et al., 2004). The secretion of salivary amylase is directly stimulated by innervation followed by hormonal regulation in response to changes in serum nor-adrenalin levels. Therefore, the salivary gland acts more quickly and sensitively responds to the psychological stress than cortisol (Yamaguchi et al., 2004). The measurement of sAA is an efficient and non-invasive assessment to study the effect of psychosocial stress. In the present study, considering possible individual variability in responding to the same stressful condition, trait anxiety, physical condition, subjective stress, achievement emotion and sleeping time were scored and integrated with the changes in sAA in each participant during pharmacy practice. Our results suggest that the theanine supplementation is beneficial in suppressing psychosocial stress in humans.

2. Methods

2.1. Participants

Twenty healthy 5th-year students of the University of Shizuoka, who participated in the experiment, were randomly divided into two groups with matching sex: theanine ($n = 10$, 7 men and 3 women; average age 22.5 ± 0.2 yr) and placebo ($n = 10$, 7 men and 3 women; average age 22.2 ± 0.1 yr) via sealed envelopes to receive theanine or placebo tablets. The students were assigned to practice outside the university, in a hospital or a drug store for 11 weeks. The first 10 days of the practice program were analyzed, because these days were assumed to be the most stressful. None of the participants indicated acute or chronic disease, regular medication intake, or habitual smoking. They were instructed to drink mainly water, and not to take theanine- and caffeine-rich beverages such as green tea, coffee, and black tea throughout the experiment. The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the University of Shizuoka. All the participants received verbal and written information about the study and signed an informed consent form before entering the study. This study was registered at clinicaltrials.gov (registration ID no. NCT01361204). The study period was from March to September in 2011.

2.2. Procedure

This study was a group comparison design and participants were randomly assigned to theanine or placebo groups. The participants did not know whether they were consuming theanine or placebo. To assess the anxiety of the participants, the state–trait anxiety inventory (STAI) test (Japanese STAI Form X-1, Sankyo, Kyoto, Japan) was carried out before the pharmacy practice.

Theanine or placebo (lactose) tablets (200 mg, twice a day, after breakfast and lunch; Lyon et al., 2011; Kimura et al., 2007; Lu et al., 2004) were taken from 1 week prior to the pharmacy practice and continued for 10 days in the practice period, in total for 17 days.

The placebo tablet of lactose was in a similar color to a theanine tablet. A questionnaire including physical condition, subjective stress and achievement emotion was assigned for 10 days after each day's practice. The physical condition of the participant was assigned an ordinal scale (5, very good; 4, good; 3, normal; 2, a little bad; 1, bad). Subjective stress was evaluated using visual analog scales (VAS: 0–10) from very relaxed to highly stressed. Achievement emotion was assigned an ordinal scale (5, completely; 4, better; 3, a little better; 2, a little worse; 1, much worse). Sleeping hours were also recorded.

2.3. Measurement of sAA

To assess the physiological stress response, sAA was measured using a colorimetric system (Nipro Co., Osaka, Japan; Yamaguchi et al., 2004). Briefly, a substrate 2-chloro-4-nitrophenyl-4-O- β -D-galactopyranosylmaltoside is hydrolyzed by salivary amylase in the presence of maltose, a competitive inhibitor. This reaction turns a color of a reagent strip from yellow to white, which change is quantified using a salivary amylase monitor. One unit activity (U) per mass of enzyme is defined as the production of 1 μ mol of the reduction sugar, maltose, in 1 min (NC-IUBNB, 1992).

Saliva was collected twice a day, in the morning after waking up and in the evening after practice, for 10 days during the practice. Prior to sampling, participants washed their mouths with water. After saliva was collected for 30 s using a sampling tip, each participant measured own sAA immediately every morning and evening for 10 days (including unassigned days, (i.e., a weekend), which measurement was excluded in the analyses).

To establish a no-stress and no-medication baseline of sAA, the participants measured sAA every morning and evening for 10 days during routine daily life at the university. The measurement was carried out before the pharmacy practice.

2.4. Statistical analysis

All results are expressed as mean \pm SEM. The influence of stress on sAA was evaluated by two-way ANOVA and the Bonferroni test for differences between means. Correlation coefficients were obtained using a statistical analysis program (StatPlus, AnalystSoft Inc., online version). The comparison of correlation coefficients between placebo- and theanine-groups was carried out using Fisher's z-test. In each analysis, a p value < 0.05 was considered to be statistically significant.

3. Results

3.1. Changes of sAA

There was no significant difference in sAA levels between in the morning and in the evening during routine daily life at the university

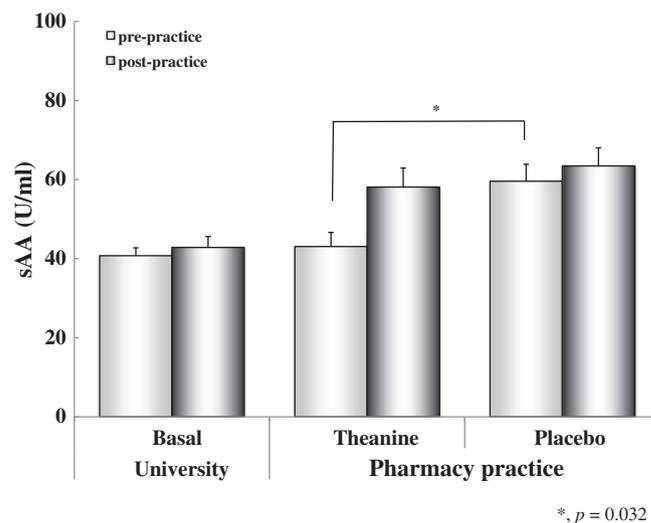


Fig. 1. Salivary α -amylase activity (sAA) of the participants during pharmacy practice was measured in the morning after waking up (pre-practice, gray bar) and in the evening after practice (post-practice, black bar). Theanine or placebo (lactose) tablets (200 mg, twice a day, after breakfast and lunch) were taken from 1 week prior to the pharmacy practice and continued for 10 days in the practice period. To assess a basal level, the participants measured sAA during daily life at the university with no-medication. The levels of sAA in unassigned days were not included in the analysis. Data are expressed as mean + SEM (*, $p = 0.032$).

Download English Version:

<https://daneshyari.com/en/article/8351661>

Download Persian Version:

<https://daneshyari.com/article/8351661>

[Daneshyari.com](https://daneshyari.com)