

WOMPERSKI, Karol, WOŹNIAK, Justyna, SZYMONIK, Julia, SZOPA, Sebastian and ELIAS, Jagoda. Benefits of l-theanine supplementation and its possible role in clinical use – review. *Quality in Sport*. 2024;15:50536. eISSN 2450-3118. DOI <https://dx.doi.org/10.12775/QS.2024.15.003> <https://apcz.umk.pl/QS/article/view/50536>

The journal has had 20 points in Ministry of Higher Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Higher Education and Science of 05.01.2024. No. 32553. Has a Journal's Unique Identifier: 201398. Scientific disciplines assigned: Economics and finance (Field of social sciences); Management and Quality Sciences (Field of social sciences). Punkty Ministerialne z 2019 - aktualny rok 20 punktów. Załącznik do komunikatu Ministra Szkolnictwa Wyższego i Nauki z dnia 05.01.2024 r. Lp. 32553. Posiada Unikatowy Identyfikator Czasopisma: 201398. Przynależność dyscypliny naukowej: Ekonomia i finanse (Dziedzina nauk społecznych); Nauki o zarządzaniu i jakości (Dziedzina nauk społecznych).
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The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 24.03.2024. Revised: 25.04.2024. Accepted: 25.04.2024. Published: 25.04.2024.

Benefits of l-theanine supplementation and its possible role in clinical use – review

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Abstract

Introduction: Green tea has been a meaningful element of various cultures among the world for ages and its benefits for human health are widely known. Discovery of its ingredients facilitated the profound research of their positive role and mechanisms of action. One of them – l-theanine - seems to have a multidirectional influence on human health.

Methods and materials: A review of chosen literature from PubMed database, GoogleScholar database between the years 2000-2023 was conducted using the following keywords: “theanine”, “theanine cognition”, “theanine brain activity”, “theanine neural activity”, “theanine neuroprotection”, “theanine stress”, “theanine cancer”, “theanine immunity”, “theanine safety”, “theanine caffeine”, “theanine metabolism”

Aim of study: Review of current knowledge on l-theanine, its benefits for health, mechanisms of action, possible applications in clinical use and safety.

Results: L-theanine has a profitable influence on cognitive performance, stress management, neuroprotection, immunity and exhibits anti-cancer properties while having virtually no detrimental side effects.

Conclusions: Further research on application of l-theanine in clinical use is necessary as studies carried out on humans included only a limited number of participants.

Keywords: “theanine”, “theanine benefits”, “theanine health”, “theanine safety”

Introduction

L-theanine also known as γ -glutamylethylamide is a non-protein amino-acid molecule naturally present in the leaves of green tea (*Camellia* sp.) and the mushroom *Xerocomus badius*. It constitutes approximately 1-2% of dry weight of tea which amounts to about 20 mg per serving (200ml). [1] In the United States it was granted a GRAS (generally recognized as safe) status by the FDA. [2] The substance is believed to bring many benefits for both somatic and neuropsychiatric health, examples of the latter being enhancing cognition, focus, sleep quality and relieving anxiety. The goal of this paper is to review the advantages and potential risks of l-theanine supplementation.

Mechanism of action

L-theanine is rapidly absorbed into the bloodstream in the small intestine through sodium-coupled active transporters. After oral ingestion the serum concentration reaches maximum

levels within 30 minutes and increases in a dose-dependent manner. It enters the brain through the blood-brain barrier. [3,4]

On a molecular level, due to its similarity to the excitatory neurotransmitter glutamate molecule, l-theanine binds to the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainite and N-methyl-d-aspartate (NMDA) glutamate receptors and acts as their antagonist. Although its binding ability is approximately 80 times lower than that of glutamate it is still pharmacologically effective [5,6]. Moreover, l-theanine increases the levels of inhibitory neurotransmitter - gamma-aminobutyric acid (GABA) and the density of its receptors. [7]

L-theanine also impacts the resting state of the brain by increasing the power of the alpha frequency band (8-12Hz) in the electroencephalogram (EEG) in the occipital and parietal regions. Historically alpha waves were believed to be the main pattern of brain activity during rest, however recent discoveries prove that they are also responsible for the state of relaxed focus. It means they facilitate concentration of the mind on a particular task at the same time attenuating the activity of the brain parts that are not in direct use at the time being. [8] In the research by Anna C Nobre et al. participants were administered 0.5mg/kg of l-theanine and their EEG activity was measured after 45 minutes and subsequently every 15 minutes. Even though the power of the alpha band increased linearly also in the control group (that may be because the participants were at rest) the enhancement was twofold higher in the experimental group. [9]

Cognition

There is evidence suggesting positive influence of l-theanine on different aspects of cognition. In the study by Hidese S. et al. cognitive functions among healthy participants were assessed with Brief Assessment of Cognition in Schizophrenia (BACS) tool which includes such parameters as verbal memory, working memory, motor speed, verbal fluency, category fluency, letter fluency, attention and executive function. After four weeks of administration of 200 mg of said substance per day improvements in verbal fluency and executive functions were observed, while score in the letter fluency was higher among participants with poorer pre-treatment performance. [10] Study by Baba Y et al. investigated cognition performance in middle-aged and older subjects using Cognitrix test. Results showed that a single dose of l-theanine may improve working memory and executive function. [11]

L-theanine has even more potent influence on cognition when combined with another ingredient of tea – caffeine. Caffeine itself is known for its pro-cognitive and attention enhancing properties, however, as the study suggests, these substances may have a synergistic mechanism of action. [12] Study by Kahathuduwa CN et al. looked at the effects of administration of 2.5 mg/kg of l-theanine and 2.0 mg/kg of caffeine (separately and in combination) among children with Attention Deficit Hyperactivity Disorder (ADHD) using the NIH Cognition Toolbox, the Go/NoGo task and the Stop-signal task. L-theanine significantly improved total cognition composite and sustained attention in the NIH Cognition Toolbox. Similarly, caffeine improved the Go/NoGo hit rate. Consequently, the l-theanine–caffeine combination improved cognition composite, sustained attention as well as sensitivity to the Go signal in the Go/NoGo task and showed a trend of improvement in inhibitory control in the Stop-signal task. Moreover the study investigated the effects of the substances on the default mode network (DMN) using functional magnetic resonance imaging (fMRI). Both substances lowered task-related brain reactivity of the DMN compared to placebo, suggesting decreased task-related mind wandering associated with ADHD. [3] Another study by Kahathuduwa CN et al. compared the effects of l-theanine (200 mg), caffeine (160 mg), their combination, black tea (one cup) and a placebo (distilled water) on cognitive and neurophysiological measures of attention. Cognition was assessed with simple (SVRT) and recognition visual reaction time (RVRT) while neurophysiologic activity with event-related potentials (ERPs) which is a measure of attention. The average RVRT was significantly improved by theanine ($P = 0.019$), caffeine ($P = 0.043$), and theanine-caffeine combination ($P = 0.001$), but not by tea ($P = 0.429$) nor by placebo ($P = 0.822$). Theanine-caffeine combination produced a significantly larger mean ERPs amplitude than placebo ($P < 0.001$), theanine ($P = 0.029$) or caffeine ($P = 0.005$), suggesting its synergistic effect on attention. [13] 2017 Kahathuduwa study looked into the visual stimulus discrimination and neural activity after the administration of 200mg of l-theanine, 160mg of caffeine, their combination or placebo. Participants had a 20-minute fMRI scan taken while performing a visual color stimulus discrimination task. Both l-theanine and l-theanine-caffeine combination were associated with faster responses to targets compared with placebo ($\Delta=27.8$ milliseconds, $P=.018$ and $\Delta=26.7$ milliseconds, $P=.037$, respectively). L-theanine produced lower neural activity to distractor stimuli in the brain regions responsible for regulating visual attention. Combination of l-theanine and caffeine resulted in lessened activity of the brain regions that typically show increased activation during mind wandering, therefore enhancing attention to target stimuli. [14] Yilmaz U et al. performed an analysis on professional curling players

investigating their shooting and cognitive performance after administration of l-theanine and caffeine separately and combined in comparison to placebo. Shooting performance was measured with the amount of points scored in an experimental competition and cognition was assessed with the Stroop test. The results have shown that shooting scores were significantly higher in the group with combined intake than the separate or placebo group. Similarly the reaction time in Stroop tests were significantly lower in the combined intake group. [15]

Neuroprotection

It has been proven in studies on cell cultures and animals that l-theanine has a neuroprotective effect on the neuronal tissue. A study by Takeshima M. et al. indicates that astrocytes are more effective in reducing neuronal loss in excess dopamine-induced neurotoxicity after being pre-treated with l-theanine. Moreover l-theanine stimulates astrocytes to synthesize glutathione (GSH) - a major antioxidant that protects neurons against oxidative stress. [16] Cho et al. reports that l-theanine shields neurons from toxicity induced by rotenone and diethyldithiocarbamate – substances suspected of being potential environmental risk factors of Parkinson's disease. [17] Song HS et al. studied l-theanine's influence on reserpine-induced orofacial dyskinesia (animal model of human tardive dyskinesia) indicating that treatment with said substance was able to prevent the symptoms of dyskinesia and reduce the reserpine-induced neuroinflammatory effects. [18] L-theanine also promotes functional recovery after spinal cord injury in rats by reducing the concentration of neuroinflammatory and apoptotic markers and increasing antioxidative potential in the spinal cord. [19] In addition, possibly due to the ability to increase GABA concentration and GABA receptors up-regulation, l-theanine has a positive impact on sleep induction and sleep duration. [7] Sleep deprivation is strongly linked to the prevalence of neurodegenerative diseases, dementia and cognition impairment. [20] In Europe almost one in three people suffer from sleeping problems, while in the USA the number is even higher reaching more than half of the society. [21] Therefore l-theanine may be a non-pharmacological aid for sleep disturbances acting as a neuroprotective factor in an indirect manner. L-theanine also protects neurons from glutamate excitatory neurotoxicity. Glutamate is one of the fundamental neurotransmitters and plays a crucial role in neuroplasticity, memory, learning and maintaining energy levels. [22] In healthy subjects glutamate is released from neurons by the transmission of an impulse and binds to the receptors responsible for opening ion channels. Afterwards it is transferred into neurons and glial cells by glutamate transporters, maintaining relatively constant concentration in the extracellular space. However, in case of ischemia or injury, due to the depletion of

intracellular energy sources of ATP and therefore depolarization of the cell membrane, excessive amount of glutamate is released, which binds to the NMDA and non-NMDA receptors causing increased membrane permeability to calcium ions. Elevated levels of calcium excessively activate the cell enzymes resulting in neuronal death. [6] Kakuda T. et al. investigated l-theanine's neuroprotective potential in the context of glutamate toxicity. Using transient ischemic model in rats they observed that premedication with l-theanine at doses of 125 μ M and 500 μ M suppressed the cell death in the hippocampus by 60% and 90% respectively. Possible explanation lies in the fact that l-theanine binds to the AMPA, kainate and NMDA receptors acting as an antagonist, therefore decreasing Ca^{2+} discharge. However, the same study showed that the affinity of l-theanine to the receptors is way lower than that of glutamate. Thus other mechanisms are most likely contributing to the neuroprotective effects. [6]

Stress management

Several studies report that l-theanine may be useful in relieving stress symptoms. Study by Hidese et al. used the Self-rating Depression Scale (SDS), the State-Trait Anxiety Inventory (STAI), and the Pittsburgh Sleep Quality Index (PSQI) to assess depression, anxiety and sleep quality, respectively, after four weeks of 200mg/day l-theanine ingestion. SDS decreased by 2.53 ($P = 0.019$) and STAI decreased by 3.37 ($P = 0.006$). In the PSQI subscales sleep latency and daytime dysfunction decreased significantly. [10] White DJ et al. reports that administration of 200 mg of l-theanine lowers the subjective stress response to a cognitive stressor one hour and salivary cortisol three hours after ingestion. [22] Research done by Michael S Ritsner et al. aimed to assess the influence of 8-weeks 400 mg/day l-theanine therapy on anxiety levels among patients diagnosed with schizophrenia using the Hamilton Anxiety Rating Scale (HARS). Compared with placebo l-theanine application resulted in greater reduction of anxiety ($P = 0.015$). [23] Another study focused on the anti-stress effect of l-theanine in 5th-year university students during pharmacy practice. Participants were ingesting 400mg/day of l-theanine one week before the practice period and 10 days into the period. The levels of stress were evaluated with the state-trait anxiety inventory test. The perception of stress was markedly decreased in the experimental group in comparison to the placebo intervention ($p=0.020$). [24] Study by Rizzo et al. investigated the effectiveness of both l-theanine and vitamin B6 in reducing tics and co-occurring disorders in a group of youth with chronic tic disorder (CTD) or Tourette syndrome with anxiety symptoms. Additionally, the study compared the results of supplementation to the effectiveness of psychoeducation in the

abovementioned disorders. Patients were assessed with the Yale Global Tic Severity Rating Scale (YGTSS) and the Multidimensional Anxiety Scale for Children (MASC) at the baseline and after 2 months. Patients were divided into two groups: “THE-group” which received supplementation with 200mg/day of l-theanine and vitamin B6 for 2 months and the “N-group” which received psychoeducation. Both groups benefited from the treatment, however the YGTSS score decreased by 43,5% in the “THE-group” and only 18,3% in the “N-group”. MASC scores decreased by 20.2% in the “THE-group” and 12,7% in the “N-group”. [25]

Anti-cancer properties

There is evidence based on research done in cell and animal models suggesting that l-theanine may have anti-cancer potential. It is confirmed that l-theanine and its derivative ethyl 6-bromocoumarin-3- carboxyl L-theanine (TBrC) inhibit cell growth and migration in highly-metastatic human cervical cancer. L-theanine and TBrC reduce the phosphorylation and expressions of EGFR, Met, Akt, and NF- κ B in cervical cancer cells by repressing the HGF- and EGF+HGF-activated EGFR/Met-Akt/NF- κ B signaling pathway. Moreover, these substances suppress the growth of human cervical tumor in tumor-bearing mice without doing any harm to the animals. [26] In addition, the study indicated that l-theanine has a potential use in the treatment of metastatic prostate cancer as it can suppress the invasion, migration, and increase cell to cell adhesion of prostate cancer cells in vitro and in vivo by significantly inhibiting the ERK/NF- κ B signaling pathway and the binding activity of p65 to the promoter regions of MMP9 and Snail. [27] L-theanine may be also helpful in the management of digestive system cancers. In a study involving thirty rats with dimethylhydrazine-induced colon cancer l-theanine reduced the number of cancerous and precancerous lesions, the volume of tumors, the Ki-67 immunostaining and the expression of Akt/mTOR and JAK2/STAT3 oncogenic pathways. [28] Furthermore l-theanine may promote apoptosis in human HepG2 hepatoblastoma cells and HeLa adenocarcinoma cells via the mitochondrial pathway. [29] Moreover, L-theanine and its semi-synthesized derivative (R)-2-(6,8-dibromo-2-oxo-2H-chromene-3-carboxamido)-5-(ethylamino)-5-oxopentanoic ethyl ester (DTBrC) also slowed down the growth and migration of human hepatocellular carcinoma (HHC) cells in in vitro, ex vivo, and in vivo HHC models. [30] A study on cell cultures also showed that l-theanine is able to attenuate proliferation and migration as well as stimulate apoptosis of melanoma cells by enhancing expression of brain and muscle Arnt-like protein 1 (BMAL1) and therefore raising the p53 transcriptional activity. [31] Liu Q et al. proved that l-theanine limits the in vitro and ex vivo proliferation of human non-small cell lung cancer A549 and

leukemia K562 cell lines and suppresses the migration of A549 cells. Additionally, l-theanine strengthened the activity of anticancer medications such as trichostatin A (the histone deacetylase inhibitor), berbamine and norcantharidin. [32] L-theanine may be a substance used in lowering the toxicity of radio- and chemotherapy. In a group of rats that underwent radiation at the dose of 5 Gy the pre-treatment with l-theanine and cystine significantly inhibited the weight loss and improved the survival rate of rats as well as lowered bone marrow cell loss and decreased the number of apoptotic cells in bone marrow. [33] L-theanine also protected male mice from cyclophosphamide-induced testicular toxicity. 5 days of pre-treatment with the amino acid resulted in reduction of testicular damage concerning spermatogonial cells, epithelial cells, seminiferous tubules and basement membrane. [34]

Immunology

There are numerous studies on animals and humans demonstrating the beneficial role of l-theanine on immunity. Hu Y et al. observed that it may inhibit the heat-induced impairment of immune system cells in mice. The treatment with L-theanine could lower the heat stress-induced reductions in body weight and food intake in mice, attenuate injury to the liver and jejunum as well as decrease the levels of the inflammatory markers (such as IL-6, IL-1 β , and TNF- α), aspartate aminotransferase and alanine transaminase activity while increasing the IgA, IgM, and IgG levels. [35] L-theanine could also protect yellow-feathered broilers from lipopolysaccharide(LPS)-induced immunological stress. Its application significantly mitigated LPS toxicity, that is decreased parameters such as food intake, body weight gain, increased mortality, elevated levels of serum cortisol, interleukin-6 (IL-6) and reduced immune contents of jejunal mucosal secretory immunoglobulin A (sIgA). L-theanine even had a better influence on body weight gain in broilers before LPS administration than antibiotics. [36] Li C et al. demonstrated that l-theanine may improve immunity in rats by altering the Th2/Th1 lymphocyte balance which could be impaired by various antigens, pathogens, cytokines etc. He found that the intragastric supplementation of L-theanine significantly reduced the ratio of Th2/Th1 in the serum of rats leading to a shift in the balance towards Th1, strengthening immunity against pathogens. In addition, a high-dose of L-theanine increased the levels of dopamine and serotonin in the pituitary and hippocampus and decreased corticosterone levels in the serum. [37] Xu Y et al. study indicated a possible application of l-theanine in psoriasis treatment. Apical administration of l-theanine in rats with Imiquimod-induced psoriasis reduced epidermal thickness and inflammatory response by decreasing the levels of IL-23 and chemokines and inhibiting the activation of NF- κ B and IL-17A signaling pathways as well as

promoting the propanoate metabolism responsible for the downregulation of the activity of TH17 cells. [38] Another study investigated the potential of l-thanine and cystine combination in boosting immunity against the influenza virus in aged mice. 14 days of pre-treatment with those amino acids before the immunization significantly increased the serum antigen-specific IgM and IgG levels in 24-month-old mice. Moreover, it restrained the infection-associated weight loss. [39]

Studies on humans are less numerous, however, there are several that are consistent with the research done on animals. Study on the Polish Rowing Team by Juskiewicz A et al. focused on the influence of l-theanine on the immune system under strenuous physical exercise. Participants were receiving 150mg/day l-theanine for 6 weeks. This period was preceded by and ended with a 2000-m test on a rowing ergometer. The abovementioned supplementation resulted in significant post-exercise decrease in IL-10 levels compared to the control group. [40] IL-10 restrains the activity of Th1 cells and its cytokines (IL-2 and IFN- γ) thus its elevated level may contribute to cellular immunosuppression. [41] Consequently the elevation in IL-10 levels is associated with the regulation of the Th1/Th2 balance towards Th2, which often occurs among athletes performing extreme physical exercise, leading to increased risk of infection, inflammation and overtraining. [42] Therefore, l-theanine, by decreasing the IL-10 concentration, may have favorable influence on the Th1/Th2 balance and immunity.

Because of the fact that l-theanine may reduce inflammation its perioperative application was also analyzed. In patients undergoing the procedure of gastrectomy the administration of l-theanine (280 mg) and cystine (700 mg) for 10 days beginning from 5 days before the procedure inhibited the postoperative increase in resting energy expenditure, the rise in interleukin-6 and C-reactive protein levels, both the lymphocyte and granulocyte count as well as prevented the increase in body temperature. [43]

Recent study also demonstrated the potential of the before mentioned l-theanine derivative – TBrC in the struggle against SARS-CoV-2 (wild-type, Delta and Omicron). TBrC interfered with the interaction of the virus spike protein and the host ACE2 receptor – the basic mechanism of SARS-CoV-2 entry into the host cells. Additionally, it hindered the binding of NF- κ B p65 to the DNA – process induced by the virus spike protein. NF- κ B p65 is one of the main transcription factors stimulating production by peripheral blood mononuclear cells and lung epithelial cells of the pro-inflammatory cytokines, such as IFN, TNF α , etc., which cause pulmonary damages. [44,45,46]

Conclusions

L-theanine seems to be a promising aid in enhancing everyday performance by augmenting cognition, attention and relieving stress, but also, even more importantly, it may be helpful in cancer treatment, reduction of inflammation and protection against nervous system impairment due to its neuroprotective properties. Nevertheless, large scale, randomized studies on humans are still necessary as those already carried out included only a limited number of participants. L-theanine may be considered safe due to the fact that none of the studies mentioned in this paper reported significant adverse reactions to the amino acid even though the administered doses were up to twenty times higher than those ingested in an average cup of green tea.

Author's contribution: All authors contributed to the article. Conceptualization – Karol Womperski; methodology – Sebastian Szopa; check - Justyna Woźniak; formal analysis – Julia Szymonik; resources – Sebastian Szopa; writing - rough preparation – Jagoda Elias; writing - review and editing - Julia Szymonik; visualization – Jagoda Elias; supervision – Justyna Woźniak; project administration – Karol Womperski. All authors have read and agreed with the published version of the manuscript.

Disclosures: No disclosures.

Financial support: No financial support was received.

Conflict of interest: The authors declare no conflict of interest.

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