

Review Article

Theanine: the unique amino acid in the tea plant as an oral hepatoprotective agent

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For thousands of years, humans have consumed tea made from leaves of *Camellia sinensis*, first as a medicinal herb and then as a widely popular beverage. In the past 10 years, theanine, a tea-derived, unique, nonproteinic amino acid, has been extensively studied for its health benefits. Recently, multiple lines of evidence have proven its beneficial effects on hepatic and immune functions. One possible mechanism for its biological activity involves the downregulation of the inflammatory response through the induction of nitric oxide production and glutathione synthesis. In this review, we summarize published results describing the potential mechanisms for these beneficial health effects and provide new insight into how theanine can be therapeutic for liver injury and chronic liver disease.

Key Words: tea plant, theanine, chronic liver disease, hepatoprotective effect, immune functions

INTRODUCTION

Tea is made from the leaves of *Camellia sinensis*, a warm-weather evergreen plant, and is one of the most widely consumed beverages worldwide. Historically, tea has been used as a medicinal herb, dating back 4700 years to China, and in Asia, tea drinking continues to be regarded as a healthy practice, especially for improving liver function. Although catechins and caffeine are the primary bioactive components considered to contribute to the beneficial effects of tea, the health benefits of theanine have also become prominent in recent years. Theanine is a unique nonproteinic amino acid found in the tea plant. The International Union of Pure and Applied Chemistry name of theanine is 2-amino-4-(ethylcarbamoyl) butyric acid.¹ Similar to other natural amino acids, theanine is a chiral species and is predominantly observed in the L-enantiomer form.² In this review, theanine will refer specifically to L-theanine. In the tea plant, theanine is biosynthesized from glutamic acid and ethylamine by the enzyme theanine synthetase.³ Theanine accounts for approximately 50% of the total amino acids in tea leaves. It comprises approximately 1%–2% of the total dry weight of green tea leaves; one cup of green tea contains approximately 8–30 mg of theanine.⁴ Theanine is readily bioavailable on consumption and is quickly absorbed in the intestinal tract, followed by metabolism in the liver.^{5,6} Acute and chronic toxicity tests conducted on the safety of theanine have not yet established a toxicity threshold.⁷ Theanine is available as a dietary supplement and has been granted the “generally recognized as safe” status by the U.S. Food and Drug Administration.

More than 10% of the global population is estimated to be affected by chronic liver diseases, which may consequently progress to fibrosis, cirrhosis, or hepatocellular carcinoma as a result of sustained inflammation and the corresponding regenerative response.⁸ In recent years, the incidence of chronic liver disease has been gradually increasing worldwide and will present an enormous problem for public health. Prevention and treatment for liver diseases has been extensively explored, with remarkable progress being achieved in recent decades; however, the availability of treatments and the outcomes of individuals with chronic liver disease are still not ideal.⁹ Therefore, there is an urgent need for effective and affordable hepatoprotective agents. Several studies, including animal studies, epidemiological studies, and human interventions, support the conclusion that tea and tea extracts have a protective effect on liver tissue.^{10–15} For example, Ruhl et al¹⁶ reported that the consumption of tea (>2 cups per day) is associated with a lower prevalence of chronic liver disease in the United States. Although most studies on tea have focused on catechins, theanine may be the compound that mediates the hepatoprotective capabilities of

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tea. Several reports have examined the hepatoprotective effects of theanine on toxicity-induced hepatic injury and its pharmacokinetic and pharmacodynamic properties.¹⁷⁻¹⁹

Pharmacokinetics and bioavailability of theanine

The bioavailability of theanine after oral consumption is relatively high. In humans, theanine is rapidly absorbed after the intake of pure theanine and green tea. Kitaka et al²⁰ reported that the intestinal absorption of theanine was mediated by a common Na⁺-coupled cotransporter in the brush-border membrane. After the oral intake of 200 mg theanine in rats, significant concentrations of theanine could be detected in the plasma, liver, and urine, and the highest plasma theanine levels were observed at approximately 0.5 h after administration.^{21,22} Scheid et al¹⁷ have reported that after the oral intake of 100 mg of pure theanine in healthy humans, the plasma concentration increased time-dependently up to 3.0 h postconsumption and returned to baseline at 24 h after intake. After oral ingestion, theanine is absorbed through the small intestine and hydrolyzed into glutamic acid and ethylamine in the intestine and liver, followed by urinary excretion.^{17,23} Orally administered theanine is thus easily absorbed from the intestinal tract and almost immediately transported into the liver and blood. Therefore, theanine is considered to function as a donor that supplies glutamic acid to the body.

Characteristics and biomarkers of hepatic injury

Hepatic injury is most commonly caused by sustained exposure of the liver to harmful exogenous entities, including alcohol, viruses, toxicants, and biotransformed metabolites, which induce chronic inflammation of the liver, leading to diseases such as fibrosis, cirrhosis, and hepatocellular carcinoma.²⁴ Fibrosis is a wound-healing process initiated by inflammation and oxidative stress and can eventually develop into hepatocellular carcinoma.²⁵ The development of chronic liver diseases is associated with the activation of the immune system, along with the upregulation of inducible nitric synthase (iNOS) and cyclooxygenase-2 (COX-2). Oxidative stress is a common etiological pathway for most liver diseases, and it is often induced by ionizing radiation, toxins, and drugs.²⁶ Reactive oxygen species (ROS) are likely produced from the cytochrome P450 2E1 (CYP2E1) and mitochondria in hepatocytes and Kupffer cells (KCs).²⁷ The liver is the main organ responsible for the homeostatic detoxification of metabolic waste products and scavenging for ROS. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR), and antioxidants, including glutathione (GSH), play an essential role in the hepatic clearance of ROS. Emerging evidence has demonstrated that hepatic injury can be produced by suppressing hepatic antioxidant enzymes.²⁸ In addition, inflammation is involved in liver diseases and hepatotoxicity, in which the activation of transcription factor nuclear factor κ B (NF- κ B)-mediated mechanism stimulates the generation of proinflammatory cytokines from KCs, including iNOS, COX-2, tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , IL-6, interferon- γ (IFN- γ), tissue growth factor- β (TGF- β), and connective tissue growth factor (CTGF).^{29,30} These proinflammatory

cytokines promote hepatic and systematic inflammation, thereby changing the microenvironment in the liver, which in turn leads to fibrosis and abnormal hepatocyte regeneration.^{30,31}

Hepatoprotective effect of theanine

To the best of our knowledge, tea is the only dietary source of theanine. Teas are classified into different categories according to their fermentation through wet-heat conditions and/or intrinsic polyphenol oxidases and peroxidases, which cause the oxidation of catechins that are abundant in fresh tea leaves. The most commonly consumed teas worldwide are nonfermented green tea and intensively fermented black tea.³² Green tea has been shown to protect against hepatic injury in various toxic murine models, including carbon tetrachloride (CCl₄),¹¹ dextran sodium sulfate,³³ microcystin,³⁴ and alcohol.³⁵ In green tea, catechins are considered to be the most important functional components for liver protection.³⁶⁻³⁸ Compared with green tea, black tea exhibits a near-complete destruction of catechins. However, black tea has also been shown to protect against CCl₄-,³⁹ aflatoxin-,⁴⁰ and pesticide-induced⁴¹ liver injury in mice. In general, theanine content seems to be minimally affected by the fermentation process because both green and black teas contain similar theanine levels. Hence, theanine is likely to be the compound responsible for hepatoprotection.

Because of the hepatoprotective effects of theanine on hepatic cell death after exposure to liver injury agents such as CCl₄, irinotecan, doxorubicin, and ethanol, the preventive effects of theanine on liver injury in mice and rats were investigated. The excessive intake of alcohol can cause increased ROS production and lipid peroxidation, as well as decreased GSH levels in the liver. Li et al¹⁹ investigated the effect of theanine on alcoholic liver injury both in vitro and in vivo. In ethanol-treated human hepatic cells, theanine significantly protected hepatocytes against ethanol-induced cell cytotoxicity and apoptosis, through the prevention of ethanol-triggered ROS and malondialdehyde (MDA) generation in L02 cells. When mice were exposed to ethanol, their levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and MDA increased, and the activities of SOD, CAT, and GR decreased. However, when theanine was administered to mice before ethanol exposure, all of the baseline functions were restored. These results suggest that theanine can prevent ethanol-induced liver injury, possibly through enhanced hepatocyte antioxidant abilities. Furthermore, Jiang et al¹⁸ reported that theanine protects against acute liver injury induced by CCl₄, through its inhibitory effects on oxidative stress-mediated inflammatory response. In a dose-dependent manner, theanine suppressed the increase in serum ALT, AST, and bilirubin levels as well as in TNF- α and IL-1 β secretion. Moreover, the expression of COX-2 and iNOS and histopathological changes induced by CCl₄ in the liver were diminished. Theanine also significantly decreased CCl₄-induced lipid peroxidation and the metabolic activation of CCl₄, through the downregulation of CYP2E1 and the elevation of hepatic GSH content and antioxidant enzyme activities. Chronic CCl₄ treatment causes liver injury, oxidative stress, and nitrosative stress. Pérez-Vargas et al⁴² investigated the hepatoprotective

tive activity of theanine against CCl₄-induced liver injury in rats. Rats were subcutaneously injected with CCl₄ (0.4 g/kg) three times per week for 8 weeks to induce chronic chemical liver injury, and theanine (8 mg/kg, 3 days per week) was orally administered for 8 weeks. Compared with the control group, the levels of serum aminotransferase and γ -glutamyl transpeptidase (γ -GT) and the degree of lipid peroxidation were significantly lower in the theanine-treated group. CCl₄ treatment promoted NF- κ B activation and increased the expression of both TGF- β and CTGF. Theanine effectively inhibited NF- κ B activation and downregulated the proinflammatory (IL-1 β and IL-6) and profibrotic (TGF- β and CTGF) cytokines. Sugiyama et al.^{43,44} evaluated the protective effect of theanine in doxorubicin-induced acute hepatotoxicity. The results showed that the serum levels of ALT and AST and the formation of hepatic cleaved caspase-3 protein in the doxorubicin-treated group were markedly increased, and these elevations were significantly attenuated by theanine. Moreover, theanine significantly inhibited the doxorubicin-induced GSH reduction in the liver. The levels of hepatic proapoptotic protein expression of Bax and Fas were increased in the doxorubicin-treated group, and these levels were restored to control levels by theanine. The results of this study suggest that the hepatoprotective effects of theanine might be derived from a mechanism involving the suppression of intrinsic caspase-3-dependent apoptotic signaling.

The results of representative studies on the hepatoprotective effects of green tea, black tea, and theanine are summarized in Table 1, together with an appraisal of the main contributions of each study. The aforementioned results indicate that theanine can be potentially included in the treatment for hepatotoxicity and for the prevention

of further hepatic injury and chronic liver diseases.

Potential mechanisms underlying the hepatoprotective effect of theanine

During liver injury, hepatic stellate cells were contracted and activated into proliferative and fibrogenic myofibroblast-like cells, leading to the decrease in NO level and hepatic cell apoptosis. A cascade of signaling and transcriptional events in stellate cells underlies the fibrogenic response to liver injury, and the regulation of NO release is a potential target for liver protection. NO plays a crucial role in the antagonism of stellate cell contraction, inhibition of platelet adhesion, and neutralization of oxygen free radicals, thus preventing cell apoptosis.⁴⁵⁻⁴⁷ Endothelial nitric oxide synthase (eNOS) controls production of NO, which is part of a negative feedback loop for blocking inflammation and injury to liver cells.^{48,49} S-nitrosylation-mediated inhibition of TNF- α and caspase activity is the most extensively characterized mechanism for the inhibition of apoptosis by NO and is likely to be effective in cells that can efficiently limit inflammation and injury.⁴⁹⁻⁵² The decreased expression of eNOS in chronic liver disease can reduce hepatic perfusion and accelerate fibrosis.⁵³ Hence, hepatic eNOS-mediated NO production can maintain normal arterial and portal venous blood flow in the liver.⁵⁵ The deficiency of eNOS-derived NO exacerbates hepatic injury, and the overexpression of eNOS-derived NO protects against hepatic injury.^{54,56-58} In rats, eNOS activity is reduced in the liver after chronic ethanol exposure, which is correlated with liver damage, especially the inflammatory activation of NF- κ B and TNF- α expression.⁵⁹ The activity and expression of eNOS is regulated by the phosphorylation state of the phosphatidylinositol 3-kinase (PI3K) and extracellular regulated

Table 1. Hepatoprotective effects of green tea, black tea, and theanine in mice and rats

Hepatic damage agent	Outcomes	References
CCl ₄	Green tea reduces the levels of ALT, AST, bilirubin and MDA, and increases the activities of SOD, GPx in mice.	11
Dextran sodium sulfate	Green tea reduces the levels of ALT and AST, and increases the expression of HO1 and HSP70 mRNA in mice.	33
Microcystin	Green tea reduces the levels of ALT, AST and bilirubin, and increases the expression of Bcl-2 in mice.	34
Ethanol	Green tea reduces the levels of ALT and triglycerides, and increases activity of alcohol dehydrogenase in mice.	35
CCl ₄	Black tea reduces the levels of ALT, AST, MDA and 8-iso-PGF2 α , and increases the activities of SOD and GSH content in mice.	39
Aflatoxin	Black tea reduces the levels of ALT and AST in mice.	40
Pesticide	Black tea reduces the levels of ALT, AST and AKP, and increases the activities of SOD, CAT, GSH, total thiol, GPx, GR and GST in mice.	41
Ethanol	Theanine reduces the levels of ALT, AST and MDA, and increases the activities of SOD, CAT and GR in mice.	19
CCl ₄	Theanine reduces the levels of ALT, AST, bilirubin, TNF- α and IL-1 β , and increases the activities of antioxidant enzymes and GSH content in mice.	18
CCl ₄	Theanine reduces the levels of aminotransferase, γ -GT, IL-1 β , IL-6, TGF- β and CTGF and the degree of lipid peroxidation in rats.	42
Doxorubicin	Theanine reduces the levels of ALT, AST and the expression of Bax and cleaved caspase-3 in mice.	43
Doxorubicin	Theanine increases the contents of hepatic glutamate and GSH in mice.	44

AKP: alkaline phosphatase; HSP70: heat shock protein 70; 8-iso-PGF2 α : 8-isoprostane prostaglandin F2 α .

protein kinase (ERK) pathway.⁶⁰⁻⁶² Overall, the activation of eNOS via a PI3K/ERK-dependent signaling pathway is associated with the alleviation of liver injury.⁶³

To verify the anti-inflammatory effects of theanine in tea, Lorenz et al⁶⁴ chose a highly fermented black tea and a nonfermented green tea and compared their effects on NO production. Chemical analysis revealed that the black tea was almost devoid of catechins. However, the black and green teas had equal potency in stimulating eNOS activity and NO production. These results suggest that the equal levels of theanine in the teas possibly played a role in the induction of NO production. Theanine can effectively promote NO production and dose-dependently activate the phosphorylation of PI3K and eNOS expression. Additionally, theanine-induced NO production is partially attenuated in the presence of a PI3K- or an eNOS-specific inhibitor and is completely abolished using eNOS siRNA in endothelial cells.⁶⁵ Consequently, the mechanism by which theanine exerts a hepatoprotective effect is likely to be associated with the activation of the PI3K/ERK/eNOS/NO pathway.

Immunomodulative effects of theanine

The liver is intimately connected to the immune system, and its functions involve the removal of harmful stimuli and self-protection against pathogens and metastatic cells alongside the toleration of self-antigens and benign foreign antigens. The innate cells (resident macrophages or KCs, dendritic cells, natural killer and natural killer T cells) and antimicrobial components (inflammatory cytokines, chemokines, acute phase proteins, and complement proteins) of the liver coordinate to achieve this critical function and eliminate invading pathogens and infected or transformed self-antigens.⁶⁶ Studies have shown that theanine and tea have favorable effects on immune function in cell cultures, animal models, epidemiological studies, and human interventions.⁶⁷⁻⁷¹ Human $\gamma\delta$ T-lymphocytes are a subset of T cells and are the first line of defense against pathogens. Ethylamine is produced by the acid hydrolysis of theanine in the gut and by enzymatic hydrolysis mediated by the amidases in the liver, which are capable of expanding $\gamma\delta$ T cells.⁷² Moreover, a previous clinical study documented that the oral consumption of theanine enhanced $\gamma\delta$ T-cell proliferation.⁷³ Therefore, theanine is considered an essential compound for teas' ability to improve immune function.

Elderly individuals are more susceptible to influenza virus infections. The oral co-administration of theanine and cystine before vaccination can enhance immune response to the influenza vaccine in elderly persons with low total protein and hemoglobin levels.⁷⁴ Moreover, the common cold is one of the most frequent illnesses caused by viral infections. Sharma et al⁷⁵ reported that compared with a placebo group, an intervention group comprising healthy male volunteers who were administered oral supplements of cystine and theanine showed a significantly decreased incidence of colds during the trial. This result was supported by another human intervention study by Singh et al,⁷⁶ who reported that volunteers who received capsules comprising green tea catechins and theanine showed significantly decreased incidence of clinically defined influenza infection (4.1%) compared with those

in the placebo group (13.1%; adjusted OR, 0.25; 95% CI, 0.07–0.76; $p=0.022$). A double-blind intervention study by Murakami et al.⁷⁷ followed 15 individuals who undertook continuous intense exercise after they were given either 700 mg of cysteine and 280 mg theanine daily or a placebo for 10 days. An increased concentration of high-sensitivity C-reactive protein and neutrophil count in the blood as well as a decreased lymphocyte count were observed in the placebo group compared with that in the cysteine and theanine group. The relationship between cysteine and theanine consumption and the resulting immunomodulative effects was reviewed, and the authors concluded that cysteine and theanine can be considered as oral immunomodulative nutrients.⁷⁸ However, additional trials in elderly individuals are needed, especially in those with hepatic immune abnormalities, to investigate the effectiveness of theanine intake in immune-related liver disease.

In addition, Kurihara et al⁷⁴ have reported that the combined administration of theanine and cystine enhanced antigen-specific IgG production and increased the IL-10/IFN- γ ratio, partly through the increase of GSH levels and type 2 T helper cell-mediated responses in older mice. Furthermore, the combined administration of theanine and cystine enhances immune function and protects against influenza virus infection, through the augmentation of GSH synthesis in older mice.⁷⁹ Recent results have shown that the daily intragastric administration of 400 mg/kg theanine solution to rats improves immune response through an increase in the splenic organ index and a decrease in the contents of IL-4/6/10 and corticosterone and the ratio of IL-4/IFN- γ in the serum.⁸⁰ Allergic diseases result from exposure to normally harmless environmental substances known as allergens. The oral administration of theanine inhibits both IgE- and non-IgE-mediated allergic responses, decreases histamine release from mast cells, and represses TNF- α and IL-1 β /6/8 secretion through the inhibition of NF- κ B and caspase-1 activation; theanine also exhibits mast cell-stabilizing capabilities in mice.⁸¹ The immunomodulative actions of theanine are therefore important for combating infections, allergic diseases, and hypersensitivity reactions.

Conclusion

Orally administered theanine, an ingredient in tea leaves, is readily absorbed and biologically available. The possible beneficial health effects of theanine consumption have been demonstrated by many animal studies and human trials. Regarding the possible mechanisms, the actions of theanine on the downregulation of the inflammatory response through the induction of NO production and GSH synthesis are likely to be critical for the prevention of hepatic diseases as well as for the improvement of immune function (Figure 1). Considering its generally safe profile, theanine can be used as a potential treatment for hepatic injury and immune-related liver diseases. Additional evidence to support the health-promoting effects of theanine when consumed at levels consistent with current dietary guidelines is anticipated. Moreover, the synergetic health effects of theanine and other major secondary metabolites in tea, such as catechins and caffeine, should be

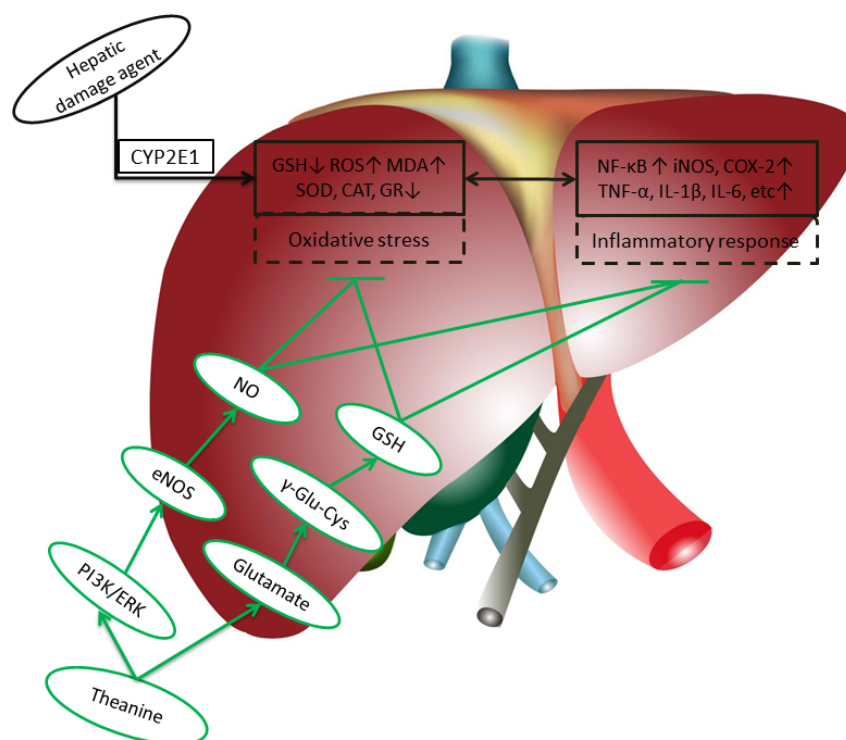


Figure 1. The putative mechanisms involved in hepatoprotection of theanine

of considerable public health interest. We anticipate that theanine will have future applications as a “food for specified health uses” as defined by the Japan Ministry of Health, Labour and Welfare and as a pharmaceutical for preventing liver injury and chronic liver disease.

AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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