JOURNAL OF FUNCTIONAL FOODS 6 (2014) 534–544

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GABA tea ameliorates cerebral cortex apoptosis and autophagy in streptozotocin-induced diabetic rats

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ARTICLE INFO

Article history:
Received 22 September 2013
Received in revised form
17 November 2013
Accepted 21 November 2013
Available online 15 December 2013

Keywords:
GABA tea
Diabetes mellitus
Apoptosis
Autophagy

ABSTRACT

The γ-aminobutyric acid (GABA) tea is popular by consumers in Asia in recent years. This study investigated the effects of GABA tea on apoptosis and autophagy in the cerebral cortex of streptozotocin (STZ)-induced diabetic rats. Thirty-four male Wistar rats at 8 weeks of age were randomly divided into control group, STZ-induced (60 mg/kg, i.p.) diabetes (DM), and DM rats with water GABA tea extracts of containing GABA either 3.01 or 30.1 μg/rat per day for 6 weeks. Treatment with GABA tea dose-dependently lowered blood glucose level in the diabetic rats compared with vehicle. GABA tea reduced the diabetic-induced Fas-dependent and mitochondrial-dependent apoptotic pathway in the diabetic cerebral cortex compared with vehicle, the evidence for which is based on decreases in Fas, activated caspase-8, pro-apoptotic t-Bid, Bax, cytosolic cytochrome c, activated caspase-9 and activated caspase-3. GABA tea also reduced the diabetic-induced autophagy. The results suggest that GABA tea obviously inhibits diabetic-induced apoptosis in the cerebral cortex through the suppression of Fas and mitochondrial pathways. These findings provide the possible diabetes-related apoptotic and autophagy pathways in the cerebral cortex and suggest that GABA tea treatment has the potential to prevent diabetic brain abnormality.

1. Introduction

Diabetes mellitus, characterized by hyperglycemia, is the most common and serious metabolic disorder. The central nervous system (CNS) complications of diabetes mellitus, diabetic encephalopathy, have been described in clinical patients and experimental models (Perros, Deary, Sellar, Best, & Frier, 1997). Diabetic encephalopathy encompasses neurophysiologic, morphological and structural changes that may lead to cognitive deficits in diabetic patients (Li, Zhang,

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Abbreviations: GABA, γ-aminobutyric acid; STZ, streptozotocin; DM, diabetes; FADD, Fas-associated death domain; C, catechin; EC, epicatechin; EGCG, epigallocatechin gallate; LC3, microtubule associated protein light chain 3

1756-4646/$ - see front matter
http://dx.doi.org/10.1016/j.jff.2013.11.020

Grunberger, & Sima, 2002). In a previous study using quantitative magnetic resonance imaging evaluation, described white matter abnormalities and brain atrophy in the diabetic mouse brain (Toth et al., 2006). The etiology of diabetic brain atrophy remains unclear. It is likely associated with neuronal loss, micro-vascular and macro-vascular complications, hypertension, and recurrent hypoglycemia/hyperglycemia (Perros et al., 1997). Tissue damage is attributed to oxidative injury resulting from the production of free radicals. Several studies have suggested that diabetic encephalopathy and brain atrophy are accompanied by neuronal apoptosis (Li et al., 2002). However, brain apoptotic pathways in diabetes are still not totally understood.

The apoptosis of hippocampus CA1 neurons has been noted in diabetics (Li et al., 2002). Apoptosis, a physiological program of cellular death, may contribute to neuronal cell loss and many neurological disorders (Culmsee & Landshamer, 2006; Nakka, Gusain, Mehta, & Raghubir, 2008), such as cerebral ischemia, Alzheimer's and Parkinson's disease (Culmsee & Landshamer, 2006). The major pathways involved in apoptotic signaling may be classified into extrinsic and intrinsic. The extrinsic Fas receptor-dependent apoptotic pathway is initiated by binding the Fas ligand to the Fas receptor. Fas receptor oligomerization recruits the Fas-associated death domain (FADD) and pro-caspase 8 to the complex and results in the activation of caspase 8, which is upstream of caspase 3, a principal effector caspase of apoptosis (Culmsee & Landshamer, 2006). Activated caspase 8 can cleave Bcl-2 homology domain 3 (BH3)-interfering domain death agonist (Bid). The cleaved Bid then causes the release of mitochondrial cytochrome c, leading to the activation of pro-caspase 9 and pro-caspase 3. The intrinsic mitochondrial-dependent apoptotic pathway is mediated by internal factors, especially in the mitochondria (Culmsee & Landshamer, 2006). The Bcl-2 family proteins reside upstream of cellular damage and focus their effects at the mitochondria (Nakka et al., 2008). Bcl-2, an anti-apoptotic protein, prevents cytochrome *c* release, whereas Bax and Bid, pro-apoptotic proteins, enhance cytochrome *c* release from the mitochondria. Cytochrome *c* activates caspase 9 and caspase 3, and executes the apoptotic program (Nakka et al., 2008).

Emerging studies also suggest that autophagy plays a pivotal role in cellular response to stress conditions (Cherra & Chu, 2008). Enhanced induction of autophagy may actively contribute to neuronal atrophy and cell death. Autophagy is a highly conserved pathway in eukaryotic organisms. Beclin-1 is involved in class III phosphoinositide 3-kinase protein complexes that regulate the initial autophagy steps and vesicle nucleation (Cherra & Chu, 2008). Atg7 and Atg12 are essential components of core autophagy machinery that regulate the vesicle elongation process (Uchiyama, Shibata, Koike, Yoshimura, & Sasaki, 2008). Membrane associated LC3, a microtubule associated protein light chain 3, is the specific marker of phagophores and autophagosomes (Towns et al., 2005; Uchiyama et al., 2008).

Tea made from the leaves of the plant *Camellia sinensis* is one of the most popular beverages consumed worldwide. Tea extract contains polyphenols (e.g., catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG)), free amino acids and caffeine. Tea has a variety of health effects, including antioxidant, anti-hypertensive, anti-arteriosclerotic and anti-diabetic activities (Anandh Babu, Sabitha, & Shyamaladevi, 2006; Hininger-Favier, Benaraba, Coves, Anderson, & Roussel, 2009; Ramadan, El-Beih, & Abd El-Ghffar, 2009; Wiseman, Balentine, & Frei, 1997). GABA tea has recently become a popular drink for health-conscious individuals in Asian countries. The steps of manufacturing GABA tea are similar to green tea, except in anaerobic incubation (Jeng, Chen, Fang, Hou, & Chen, 2007). This anaerobic process leads to high GABA and alanine but low glutamic and aspartic acids contents in GABA tea (Liu, Zhao, & Yu, 2011; Wang, Tsai, Lin, & Ou, 2006). Most of the other compounds, such as theanine, threonine, valine, methionine, tryptophan, crude fat, total free amino acids, total nitrogen, caffeine and all fatty acids did not differ between GABA and green teas (Wang et al., 2006). For catechins, GABA tea contained a similar amount of polyphenolic compounds, but total catechin, EC and EGCG were lower in GABA tea than green tea (Wang, Chuang, Hsiao, & Cherng, 2011; Wang et al., 2006). In recent years, a growing number of studies have shown that green tea can afford significant protection against various neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and ischemic damage (Mandel, Weinreb, Amit, & Youdim, 2004). GABA tea contains a high level of GABA, at >150 mg GABA/100 g on a dry weight of tea (Hininger-Favier et al., 2009; Zhao et al., 2011). It has been shown that GABA tea, like other teas, has multiple health effects, such as anti-apoptosis, antioxidant, antihypertensive and hypoglycemic activities (Abe et al., 1995; Wang et al., 2011; Wiseman et al., 1997). Recently, GABA tea has also been shown to help sleep (Cheng & Tsai, 2009). GABA is a major inhibitory neuro-transmitter in the mammalian brain and helps keeping nerve cells firing normally. Moreover, many studies suggested that impaired GABA-mediated inhibition may trigger a cascade of events leading to neuronal damage and cell death (Antony, Kumar, Kuruvilla, George, & Paulose, 2010). Under diabetic and hyperglycemia conditions, a decreased GABA uptake (Duarte, Santos, Seica, & Resende de Oliveira, 2000) and reduced extracellular GABA were observed which exacerbates neuronal injury by reducing the GABA-mediated inhibitory activity. Gabapentin, a GABA analog, is used to treat diabetic neuropathy (Baydas, Sonkaya, Tuzcu, Yasar, & Donder, 2005). Besides activation of GABA receptors can balance excessive glutamatergic excitation, inhibits the apoptosis after cerebral ischemia (Zhang et al., 2007). However, it remains unclear whether GABA tea exerts its hypoglycemic and anti-apoptotic effects in rat brain cerebral cortex when given to diabetic animals.

According to these findings, mechanisms have been proposed to explain the pathophysiology of diabetic encephalopathy, including decreased GABA neurotransmission, free radical formation, and apoptosis. GABA tea is highly enriched in GABA and tea polyphenols. Therefore, we hypothesize that GABA tea may exert a neuro-protective effect in diabetic brain against apoptosis. The purpose of our study was to examine the mechanisms and effects of chronic GABA tea supplementation on neuronal apoptotic and autophagy pathways in the brain of STZ-induced diabetes mellitus rats.