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Chronic benzodiazepine suppresses translocator protein and elevates amyloid β in mice

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ABSTRACT

Benzodiazepine (BZD) is a commonly prescribed anxiolytic and sedation aid medication, especially in elderly women. However, long-term use of BZD provokes adverse nontherapeutic effects that include movement deficit. Here, we investigated motoric deficit and molecular changes in cerebellum associated with the chronic use of BZD (cBZD) in female mice. We measured neuroprotective translocator protein (TSPO), neurotoxic amyloid β (Aβ), Aβ-producing presenilin-1 (PS1), and Aβ-degrading neprilysin. We also tested whether cBZD treatment damages mitochondrial membranes by measuring mitochondrial membrane swelling and mitochondrial respiration. Young and old mice received BZD (lorazepam) for 20 days, were tested for motoric function using Rotarod, and then euthanized to collect cerebellum. The major methods were immunoblot and RT-PCR for TSPO, PS1, and neprilysin expressions; ELISA for Aβ level; spectrometry for mitochondrial membrane swelling; XF-respirometry for mitochondrial respiration. cBZD-treated old mice showed poorer motoric function than old control or young cBZD-treated mice. Old mice treated with cBZD showed a decrease in TSPO and neprilysin and an increase in Aβ and PS1 production compared to old control mice. Old cBZD-mice also showed an increase in mitochondrial membrane swelling and a decrease in mitochondrial respiration. These data suggest that cBZD exacerbates motoric aging in a manner that involves diminished TSPO, elevated Aβ, and mitochondrial damage.

1. Introduction

The deterioration of motoric functions is one of the most common problems in elderly people, affecting daily activities and often leading to the loss of independence, and in some cases, even death (Verhaeghe et al., 1996; Cumming and Le Couteur, 2003). Such effects of age on motoric function are already burdensome, and thus, additional conditions that suppress motoric functions would be devastating. The additional conditions can be unintentionally created by prescription drugs such as benzodiazepine (BZD). BZD is a frequently prescribed CNS depressant to treat anxiety or insomnia especially for elderly (Hanlon et al., 2001; Bogunovic and Greenfield, 2004; Halme et al., 2013). Although BZDs are powerfully effective, they cause nontherapeutic effects that include movement deficit. BZD use is associated with fall injuries (Landi et al., 2005), slow motor reaction, and the inaccuracy of motor tasks (Cumming and Le Couteur, 2003; Dawson et al., 2008), all of which are more severe in the elderly than in young individuals (Greenblatt et al., 1991; Fernandez-Guasti and Martinez-Mota, 2003).

The adverse effect of BZD is particularly problematic for elderly women because they are the predominant BZD users, even when several factors are normalized (Blazer et al., 2000; Verhaeghe et al., 1996; Simon and Ludman, 2006). Elderly women are twice as likely to use BZD as elderly men (Nurmi-Luthje et al., 2006). They also significantly outnumber elderly men for lengthy BZD use (Gray et al., 2003; Wagner et al., 2004), further increasing the risk of motoric deficit (Buffett-Jerrott and Stewart, 2002; Verster et al., 2002). Despite such problems, how the chronic use of BZD (cBZD) deteriorates the motoric function of elderly remains largely unexplored. The absence of such information would continue to challenge the clinical use of BZD which is otherwise a powerfully effective medication.

BZD exerts its therapeutic effect through the CNS inhibitory γ-aminobutyric acid/BZD receptor complex (Roy-Byrne, 2005). Distinct from this, BZD binds to a mitochondrial membrane protein (Anholt et al., 1986; Basile and Skolnick, 1986), namely translocator protein

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β; cBZD, chronic benzodiazepine; MMS, mitochondrial membrane swelling; TSPO, translocator protein; PS1, presenilin-1; ROS, reactive O_2 species

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(TSPO). TSPO was initially named peripheral benzodiazepine receptor based on its expression in peripheral tissues such as kidney (Braestrup and Squires, 1977). Later on however, many studies have located TSPO in the brain (Casellas et al., 2002) and the majority of TSPO are located in the outer membranes of mitochondria (Basile and Skolnick, 1986). The biological function of TSPO appears to regulate mitochondrial homeostasis (Gatliff and Campanella, 2016), immune functions (Waterfield et al., 1999; Torres et al., 2000), and cell survival (Repalli, 2014). Especially, TSPO exerts the protective effect on brain under the pathological conditions such that treatment with TSPO ligand mitigated brain injury upon cerebral hemorrhage in mice (Li et al., 2017).

Particularly relevant to the current study is the involvement of TSPO in brain aging. The binding activity of TSPO agonist to peripheral benzodiazepine receptors was increased in the brain of Alzheimer's disease (AD) patients (Versijpt et al., 2003; Gulyas et al., 2011). AD is an age-related CNS disorder, and characterized as the accumulation of neurotoxic amyloid β (Aβ) peptide that forms plagues between neurons and inhibits neuronal functions. Aβ is produced by the enzyme complex γ-secretase, and preseniline-1 (PS1) is a major component of γ-secretase. Aβ is subject to proteolytic degradation that is mediated by Aβ-degrading proteases such as neprilysin (Shirotani et al., 2001). Accordingly, the excessive PS1 and insufficient neprilysin are associated with the accumulation of Aβ (Li et al., 2011; Hafez et al., 2011). TSPO was co-localized with Aβ in age-related CNS disorders (Cagnin et al., 2001; Ji et al., 2008) and its binding activity was also increased with age in non-demented elderly (Kumar et al., 2012). Considering the neuroprotective effect of TSPO, the increased TSPO binding activity may reflect its attempt to cope with cellular distress from brain disorders or aging.

In the current study, we were interested in identifying whether TSPO and Aβ are involved in a deleterious interaction between cBZD and age at the motoric level. We employed the chronic regimen of BZD treatment in order to model the long-term use of BZD in elderly humans. We used cerebellar tissues because this brain area controls movement and balance (Barinaga, 1996). We also investigated whether cBZD triggers mitochondrial membrane and functional damage, well-documented factors of an aging process. We report that cBZD treatment to old mice suppresses TSPO, neprilysin, but induces Aβ42 and PS1 production. These adverse biochemical changes are accompanied by mitochondrial and motoric deterioration.

2. Methods

2.1. Materials

Major analytic reagents were purchased from Qiagen (Valen-cia, CA), Sigma Aldrich (St. Louis, MO), Abcam (Cambridge, MA), EMD/Millipore (Billerica, MA), Cell Signaling (Danvers, MA), ThermoFisher (Waltham, MA), and Seahorse Bioscience, (North Billerica, MA).

2.2. Animals

Female C57BL/6 mice were obtained from National Institute on Aging. They were three (called young mice) and 16 month old (called old mice) when BZD administration began. All animals were housed at 22–25 °C and 55% humidity, with ad libitum access to water and a 12-hour light/dark cycle. Animal experimentation was conducted in accordance with the Guide to the Care and Use of Laboratory Animals [DHHS/NIH 85-23, 1996, Office of Science and Health Reports, DRR/NIH] and was approved by the University of North Texas Health Science Center Animal Care and use committee.

2.3. Animal groups and BZD injection

Young and old mice were divided into control and cBZD groups. BZD (1 mg/kg of lorazepam) or vehicle solution (0.5% methylcellulose) was intraperitoneally (IP) administered once a day for 20 days.

We mainly used old mice to model human elderly except for a behavioral test.

2.4. Accelerating Rotarod

Rotarod method measures the ability of movement and balance, and these functions are controlled by the cerebellum (Forster et al., 1996). The motor driven treadmill (Omnitech Electronics, Columbus, OH) records how quickly animals fall from an accelerating rod; a shorter latency to fall indicates poorer motor performance. The rotor consists of four cylinders that are mounted 35.5 cm above a padded surface. Mice were placed on the cylinder and a timer switch was simultaneously activated to rotate the cylinders until 44 rpm for maximum 90 s. Or when animals fell to the surface, the timer simultaneously stopped. Mice were tested for 3 sessions/day for 20 days with a 20 min resting period between sessions.

2.5. Collection of cerebellum

Next morning after all Rotarod tests, mice were euthanized under anesthesia via the drop-box method using isoflurane. A piece of gauze was placed in the 50 ml size conical tube and added with isoflurane (2 ml, 99.9%). The conical tube and mice were placed into a large container and sealed for 2 min. When mice were unresponsive to the pinch test, they were decapitated and cerebellum was collected for all biochemical studies.

2.6. Immunoblotting for TSPO, PS1, and neprilysin

The effects of cBZD on the protein contents of TSPO, neprilysin, and PS1 in mouse cerebellum were assessed by immunoblotting. Cerebellar biopsies were sonicated in lysis buffer (10 mM Tris,100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.5% deoxycholate).

(Thermo Fisher Scientific, Dallas, TX). Total protein concentration was determined in an aliquot of homogenate by Bradford assay (Bio-Rad, Hercules, CA). Another aliquot was combined with an equal volume of loading buffer (3 ml 20% sodium dodecyl sulfate, 3.75 ml 1 M Tris buffer, 9 mg bromphenol blue, 1.16 g dithiothreitol, 4.5 ml glycerol, dH20) (Thermo Fisher Scientific, Dallas, TX), denatured by heating at 90–100 °C for 10 min, electrophoresed on 4–20% SDS-PAGE, and electrophoretically transferred onto a polyvinylidene fluoride membrane. Nonspecific binding sites were blocked with 5% fat-free milk for 1 h at room temperature. The blot was washed in TBST and incubated overnight with rabbit monoclonal antibody against TSPO, PS1, and neprilysin (Abcam, Cambridge, MA) at 4 °C. The blot was then washed and incubated with horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA) for 1–2 h at room temperature. Bands were detected using the UVP (Upland, CA) luminescence system and quantified by Image J densitometry. β-actin immunoblots (Santa Cruz Biotechnology, Dallas, TX) provided loading controls.

2.7. Real-time polymerase chain reaction assay for mRNA of TSPO, PS1, and neprilysin

The messenger RNA (mRNA) abundance of PS1 was analyzed to determine if cBZD affects PS1 at the transcriptional level using a method of real-time polymerase chain reaction (RT-PCR). Total RNA was isolated from cerebellum using RNeasy spin columns (Qiagen, Valen-cia, CA) following the manufacturer's instructions. RNA was converted to cDNA by using high capacity reverse transcriptase kit (Thermo Fisher, Waltham, MA). cDNA was then quantified with a Nanodrop 2000 spectrophotometer (Thermo Fisher, Waltham, MA). RT-PCR was conducted to analyze mouse TSPO, PS1, and neprilysin gene