Toxicology and Applied Pharmacology 338 (2018) 159–173

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Anxiolytic- and antidepressant-like effects of Silymarin compared to diazepam and fluoxetine in a mouse model of mild traumatic brain injury

Morteza Kosari-Nasab[a], Ghaffar Shokouhi[a,b,c,*], Amir Ghorbanihaghjo[a,d], Mehran Mesgari Abbasi[a], Ali-Akbar Salari[a,e,*]

[a] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Department of Neurosurgery, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Neuroscience Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[e] Salari Institute of Cognitive and Behavioral Disorders (SICBD), Alborz, Iran

ARTICLE INFO

Keywords:
Depression
Anxiety
Traumatic brain injury
Inflammation
Silymarin

ABSTRACT

Clinical and experimental studies have shown that mild traumatic brain injury (mTBI) is associated with increased anxiety- and depression-related behaviors and inflammation in the brain. Unfortunately, there are no specific therapies for long-term behavioral consequences of mTBI. This study set out to determine whether silymarin treatment compared to diazepam (DZP) and fluoxetine (FLX) can reduce neuroinflammation, anxiety- and depression-like behaviors after mTBI induction in mice. We used open field, elevated plus maze, light-dark box, zero maze, sucrose preference, forced swim, and tail suspension tests to assess anxiety and depression-like behaviors in mTBI-induced mice. The levels of tumor necrosis factor (TNF)-α protein, a marker of inflammation, in the prefrontal cortex and hippocampus was also measured. This study identified that the long-term treatment with DZP, FLX or SIL results in decreased anxiety and depression-like behaviors in mTBI-induced mice. The results also showed that these drugs reduced TNF-α levels in the prefrontal cortex and hippocampus. In addition, there were no significant differences between the effects of SIL and DZP or SIL and FLX on behavioral and cytokine levels in mTBI-induced mice. Our findings support the idea that mTBI could be a risk factor for anxiety- and depression-related disorders and neuroinflammation in the brain. Taken together, this study demonstrates that DZP, FLX or SIL can significantly reduce anxiety- and depression-like symptoms, and neuroinflammation after mTBI induction in mice.

1. Introduction

Mild traumatic brain injury (mTBI) induced by external physical forces on the brain is known as a complex pathophysiological injury leading to a significant disruption of brain function (Zetterberg et al., 2013). Mild traumatic brain injury constitutes at least 80% of traumatic-related brain injuries (Kraus and McArthur, 1999). The majority of individuals with mTBI recover within a few days or weeks following initial injury (Webbe and Barth, 2003). However, recent evidence suggests that mTBI is a risk factor for cognitive dysfunctions and neuropsychiatric disorders that may appear months or even years after initial trauma (Silver et al., 2009; Mouzon et al., 2014). Although most mTBI patients will recover well, even without specific interventions (Dikmen et al., 2001; Hessen et al., 2007), some of them develop generalized anxiety (Moore et al., 2006; Gaylord et al., 2008), depression (Silver et al., 2009) and significant disability (Vanderploeg et al., 2007). Major depression and anxiety-related disorders seem to be two of the most frequent psychiatric complications among patients who experienced TBI (Moore et al., 2006; Gaylord et al., 2008; Silver et al., 2009), which are mediated by brain regions including prefrontal cortex (Meyer et al., 2012) and hippocampus (Fenn et al., 2015). Given that these disorders are associated with increased anger, aggression, the risk of suicidality, and cognitive dysfunction in persons with mild or severe TBI (Uomoto and Esselman, 1995; Hibbard et al., 1998; Fann et al., 2001; Rapoport et al., 2005), these diseases are serious public health issues which can considerably affect quality of life in humans.

Multiple lines of evidence suggest that major depression and TBI are associated with increased inflammation in the brain and blood (Morganti-Kossmann et al., 2002; Schiepers et al., 2005; Raison et al., 2006; Dowlati et al., 2010; Loftis et al., 2010; Ziebell and Morganti-Kossmann, 2010; Felger and Lotrich, 2013; Woodcock and Morganti-Kossmann, 2013). A significant increase in the levels of tumor necrosis

* Corresponding authors at: Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
E-mail addresses: shokouhigh@yahoo.com (G. Shokouhi), aa.salari@yahoo.com (A.-A. Salari).

https://doi.org/10.1016/j.taap.2017.11.012
Received 1 September 2017; Received in revised form 15 November 2017; Accepted 17 November 2017
Available online 22 November 2017
0041-008X/

factor (TNF)-α, a marker of inflammation, in the brain and blood has been reported in major depression, anxiety-related disorders and TBI (Baratz et al., 2011; Kaster et al., 2012; Chio et al., 2013; Babri et al., 2014b; Tuttolomondo et al., 2014; Majidi-Zolbanin et al., 2015; Majidi et al., 2016; Ayatollahi et al., 2017). These findings demonstrate that TNF-α plays an important role in the pathology of depression anxiety and TBI. Despite the prevalence of depression and anxiety following TBI, there is no solid evidence base within the literature for treating these disorders in this population. For instance, depression and anxiety in patients with TBI are undertreated with counseling or chemical drugs such as sertraline and fluoxetine (FLX) (Varney et al., 1987; Fann et al., 2000; Soo and Tate, 2007; Bombardier et al., 2010). Effective treatments for psychiatric diseases following TBI must be developed, tested and disseminated (Fann et al., 2015). Recent TBI literature concluded that selective serotonin reuptake inhibitors (SSRIs) like FLX appear to be the first-line agents for treatment of depression (Warden et al., 2006); besides Benzodiazepines like diazepam (DZP) are wieldy used to treat anxiety symptoms in humans (Ravindran and Stein, 2010). However, there is no high-quality studies of the treatment trials for these disorders in patients with TBI. Clinical studies suggest people with TBI may be more susceptible to the side effects associated with many psychotropic drugs (Alderfer et al., 2005; Arciniegas et al., 2005; Arciniegas and Silver, 2006). To date, no specific medications have been approved by the related agencies in different countries for the treatment of anxiety and depression induced by TBI, and finding new drugs could be very valuable. There is increasing evidence from clinical and experimental studies that Silymarin (SIL), a mixture of flavonolignans including Silybin, Silydianin and Silychristin extracted from milk thistle (*Silybum marianum*) can be used as a antioxidative, anti-inflammatory and neuroprotective drug (Gupta et al., 2000; Chtourou et al., 2010; Shaker et al., 2010). Recently, antidepressant and anxiolytic effects of SIL have been reported in rodents (Solati et al., 2012; Khoshnoodi et al., 2015; Thakare et al., 2016; Thakare et al., 2017).

Therefore, the present study was aimed to investigate the effects of SIL on mTBI-induced anxiety and depressive-like behaviors in mice. To understand whether anxiolytic and anti-depressive actions of SIL are associated with alteration of TNF-α levels in the prefrontal cortex and hippocampus, these specific brain regions implicated in pathophysiology of anxiety and depression (Drevets, 2000; Davidson, 2002; Campbell and MacQueen, 2004; Engin and Treit, 2007; Solati and Salari, 2011) were evaluated.

2. Materials and methods

2.1. Animals

NMRI mice (10–11 weeks) were obtained from the animal house of Pasteur Institute (Alborz, Iran). Animals were maintained under standard laboratory conditions on a 12:12 h light/dark cycle (lights on at 08:00 AM) and controlled temperature (23 ± 1 °C). Food and water were also available ad libitum. All procedures were approved by the Research and Ethics Committee of Tabriz University of Medical Sciences.

2.2. Experimental design

A timeline diagram of the experiments is shown in Fig. 1. In the present study, there were four experiments: In experiment 1, we investigated the effects of short-term DZP and SIL exposure on anxiety-like behavior in the open field and elevated plus maze; In experiment 2, we studied the effects of long-term DZP and SIL treatment on anxiety-like behavior in the light-dark box and elevated zero maze, and on the levels of TNF-α protein in the prefrontal cortex; In experiment 3, we evaluated the impacts of short-term FLX and SIL administration on depression-like behavior in the sucrose preference test and forced swim test; In experiment 4, we assessed the impacts of long-term FLX and SIL treatment on depression-like behavior in the sucrose preference test and tail suspension test, and on the levels of TNF-α protein in the hippocampus. In each experiment, the animals were divided into six groups (N = 10/group): Control (CON), DZP (3 mg/kg/day) (Busse et al., 2004; Violle et al., 2006) or FLX (10 mg/kg/day) (Jain et al., 2001; Yirmiya et al., 2001), SIL (50 mg/kg/day) (Soon et al., 2004; Toklu et al., 2008; Khoshnoodi et al., 2015), mTBI, mTBI + DZP or mTBI + FLX and mTBI + SIL. Animals received the drugs once daily for 20 days by gavage. The dosage of each drug was calculated at two-day intervals according to the average body weight of the animals. All drugs were dissolved in polyethylene glycol (PEG; 50%), and the controls received the vehicle.

2.3. Mild traumatic brain injury

A concussive head trauma device was used to induce mTBI in mice (Baratz et al., 2011; Zohar et al., 2011). Prior to the injury, the animals were anesthetized with an intraperitoneal injection of ketamine hydrochloride (50 mg/kg; Alfasan, Woerden-Holland) plus Xylazine (5 mg/kg; Alfasan, Woerden-Holland). The mouse head was placed directly under a hollow metal guide tube (inner diameter 13 mm). A 50-g metal weight was used to deliver the impact to the right temporal area between the corner of the eye and the ear. The weight was vertically dropped from 80 cm height down the tube to strike the skull. The head was manually supported by a sponge to avoid head movement at the moment of the impact. After the injury, the mice were allowed to recover from anesthesia on a warming pad and then placed back in their home cages. All control mice received the same procedure without being injured.

2.4. Behavioral apparatus

The observers blind to the treatment recorded all parameters for each of the behavioral tests by using a stopwatch. In addition, the tests were conducted in a quiet room during the light period (between 12:00–16:00 h) under illumination of 75 lx (Salari et al., 2016). Animals were kept in the room for at least 1 h before the assessment.

2.4.1. Open field

The open field apparatus consisted of a white wooden box (40 × 40 × 20 cm) with 16 squares (10 × 10 cm; 12 outer and 4 inner) which was directly illuminated by a 100 W bulb placed 90 cm above the center of the apparatus floor. The test period was initiated when a single mouse was placed in the middle of the apparatus and allowed to move freely for 5 min. The inner zone time in and entries (an entry was defined as all four paws) were recorded as indices of anxiety-like behavior (Babri et al., 2014a).

2.4.2. Elevated plus-maze

The elevated plus maze was performed as previously described (Enayati et al., 2012). Mice were placed individually at the center of apparatus, facing one of the open arms. The observers measured: open arm time, closed arm time, open arm entries and closed arm entries during the 5-min test period. A mouse was considered to be on the open or closed arms whenever all four paws were in the respective arm. For the purpose of analysis, open arm time % [open arm time / open arm time + closed arm time × 100], and open arm entries % [open arm entries / open arm entries + closed arm entries × 100]. The total number of open arms entries, as well as the total number of closed arm entries were defined as an index of general locomotor activity.

2.4.3. Light-dark box

The light-dark box test was performed as previously described (Salari et al., 2015). To start the test, each mouse was placed at the center of the light compartment, facing away from the door. The animal was allowed to explore freely both compartments for 5 min. The