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Effects of indole-3-carbinol on clonidine-induced neurotoxicity in rats: Impact on oxidative stress, inflammation, apoptosis and monoamine levels

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ABSTRACT

The relationship between inflammation, oxidative stress and the incidence of depression had been well studied. Indole-3-carbinol (I3C), a natural active compound found in cruciferous vegetables, was shown to have anti-oxidant and anti-inflammatory activities. Therefore, the aim of this study was to investigate the potential protective effects of I3C against clonidine-induced depression-like behaviors in rats. Also, the possible mechanisms underlying this neuroprotection; anti-oxidant, anti-inflammatory as well as the modulatory effect on monoamine levels in brain tissues were investigated. I3C was given orally (50 mg/kg) daily over 2 weeks starting 7 days before giving clonidine (0.8 mg/kg i.p.). Fluoxetine was used as a standard anti-depressant. Open-field test and forced swimming test were carried out to assess exploratory activity and despair behavior, respectively. I3C showed a significant improvement in the behavioral changes induced by clonidine. As indicators of oxidative stress, clonidine induced a significant reduction in GSH and SOD levels as well as an increase lipid peroxidation level. Tissue levels of pro-inflammatory and apoptotic markers were significantly increased in clonidine group. In addition, monoamine levels; noradrenaline and serotonin, showed a drastic decrease in clonidine group. Also, neuron specific enolase (NSE) was significantly elevated in clonidine group. In contrast, I3C pre-treatment significantly attenuated clonidine-induced oxidative stress, inflammation, apoptosis, decreased NSE expression and increased levels of monoamines. Fluoxetine was used as a standard. In conclusion, the findings of this study suggest that I3C protects against clonidine-induced depression. This neuroprotective effect is partially mediated by its anti-oxidant, anti-inflammatory and anti-apoptotic activities as well as elevating monoamines levels.

1. Introduction

Depression is a multi-causal and life-threatening psychiatric disorder which affects up to 21% of the population worldwide (Maes et al., 2009; Nemeroff, 2007). Depression was shown to be associated with high morbidity and mortality as there is a close relationship between depression and other diseases such as cardiovascular diseases, obesity, diabetes, epilepsy as well as cancer (Lang and Borgwardt, 2013).

Depression pathogenesis is thought to be complex and multi-factorial. For the last decades, the monoaminergic hypothesis of depression had been thought to be the dominant cause of depression and several anti-depressant agents were produced based on this hypothesis. However, serious limitations to the current hypothesis have emerged suggesting that other mechanisms may be involved in the pathophysiology of depression (Lang and Borgwardt, 2013). Recently, it was shown that enhanced neurodegeneration associated with depression may be partially attributed to oxidative stress and inflammation (Makhija and Karunakaran, 2013; Maes et al., 2012; Catena-Dell'Osso et al., 2011; Lucca et al., 2009; Maes et al., 2009). Reactive oxygen species (ROS) and many inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) have been shown to be involved in depression both in animal models (Lopresti et al., 2012) and in patients with depression (Rawdin et al., 2013). Moreover, the modulatory effect of desipramine on noradrenergic transmission is partially mediated by reducing TNF-α level (Reynolds et al., 2005). Therefore, other strategies beyond serotonin and noradrenaline reuptake inhibition such as

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anti-oxidative and anti-inflammatory agents may represent promising anti-depressant therapies. In this context, many anti-oxidant and anti-inflammatory agents have shown anti-depressant activity in animal models of depression (Lopresti et al., 2012; Yi et al., 2010; Kulkarni et al., 2009).

Indole-3-carbinol (I3C) is a naturally occurring compound found in cruciferous vegetables (Aggarwal and Shishodia, 2006). Many pharmacological studies demonstrated that I3C possess chemopreventive (Parkin and Malejka-Giganti, 2004) and anti-tumor activities (Abou El Naga et al., 2013; Jeong et al., 2011; Marconett et al., 2011). In addition, anti-oxidant and anti-inflammatory activities of I3C had been described in a variety of animal models (El-Shinnawy et al., 2014; Aggarwal and Shishodia, 2006). I3C was shown to inhibit cyclooxygenase-2 and lipoxygenase expression (Aggarwal and Shishodia, 2006). Also, Jiang et al. (2013) showed that I3C suppressed immune cells infiltration and pro-inflammatory cytokine production such as IL-1β, IL-6 and TNF-α in a mouse model of lipopolysaccahride-induced acute lung injury. Interestingly, it was found that I3C induced a significant reduction of inducible nitric oxide synthase (iNOS) expression in brain tissues in rats (Rostoka et al., 2010).

Therefore, the aim of this study was to investigate the potential protective effects of I3C against clonidine-induced depression-like behaviors in rats. Also, the possible mechanisms underlying this neuroprotection; anti-oxidant, anti-inflammatory, anti-apoptotic as well as the modulatory effect on monoamine levels in brain tissues were investigated. In addition, the effect on the neuronal injury marker, neuron specific enolase, was assessed

2. Materials and methods

2.1. Material

Clonidine hydrochloride and I3C were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Clonidine was used to induce depression-like behavior according to previous studies (Iwasaki et al., 2006; Srivastava and Nath, 2000; Prasad and Shotliff, 1993; Enginar and Eroglu, 1990; Parale and Kulkarni, 1986; Ortmann et al., 1982). Fluoxetine hydrochloride (FLX) was purchased from MISR Co. for Pharmaceutical and Chemical Industries (Cairo, Egypt) and it was used at concentration of 20 mg/kg orally (Jindal et al., 2013). Concentration of the drug used was selected as previously reported (Rostoka et al., 2010) as well as from pilot experimental trials of the present study. Reduced glutathione (GSH), Ellman's reagent [5,5-dithio-bis (2-nitrobenzoic acid); DTNB], bovine serum albumin and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). N-butanol, dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4) and Trichloroacetic acid (TCA) were purchased from El-Nasr Chemical Co. (Egypt). All chemicals and solvents were of highest grade commercially available.

2.2. Experimental animals

Ethical guidelines were followed according to those stated by Ain Shams University Review Committee for the use of animal subjects. Male albino rats (150–200 g) were obtained from Nile Co. for Pharmaceutical and Chemical industries, Egypt. Rats were housed in air-conditioned atmosphere, at a temperature of 25 °C with alternatively 12 h light and dark cycles. Animals were acclimatized 2 weeks before experimentation. They were kept on a standard diet and water ad libitum. Standard diet pellets (El-Nasr, Abu-Zaabal, Egypt) contained not less than 20% protein, 3.5% fat, 6.5% ash and a vitamin mixture.

2.3. Experimental design

Animals were divided randomly into five groups (eight animals per group) and treated for two weeks as follows; the first group was given the vehicle (corn oil) orally once daily. The second group was given corn oil orally once daily for the two weeks starting one week before giving clonidine (0.8 mg/kg i.p.) once daily over the second week. The third group was given I3C at a dose of 50 mg/kg dissolved in corn oil once daily for two weeks starting one week before giving clonidine (0.8 mg/kg i.p.) once daily over the second week. The fourth group was given I3C at a dose of 50 mg/kg dissolved in corn oil once daily over the two weeks. The last group served as a standard group and was given FLX at a dose of 20 mg/kg orally for two weeks starting one week before giving clonidine (0.8 mg/kg i.p.) once daily over the second week. At the end of the two weeks, behavioral tests were carried out and animals were sacrificed and the hippocampus tissues were dissected and washed with ice-cold saline. Hippocampus tissues were homogenized in saline then homogenates were used to assess oxidative stress markers (reduced glutathione (GSH), lipid peroxides and superoxide dismutase (SOD)), inflammatory mediators (IL-1β and TNF-α) as well as monoamines levels (noradrenaline and serotonin). In additions, specimens from the whole brain areas from different treated groups were taken for histopathological examination. Immunohistochemical staining was used to detect the expression of apoptotic markers (caspases-3 and cytochrome c) as well as neuron specific enolase (NSE), a neuron injury marker.

2.4. Behavioral assessment

2.4.1. Assessment of exploration behavior using open-field test (OFT)

OFT is one of the most important tests applied in behavioral studies. One day before the end of the experiment, the test was carried out where rats from different treated groups were placed into the central square of the open-field and observed over 5 min. Behavioral parameters to assess exploration (latency period, ambulation frequency and number of rearing) as well as emotionality (self groomings and defecation) were recorded (Sen et al., 2007; Bronikowski et al., 2001; Katz et al., 1981; Cunha and Masur, 1978).

2.4.2. Assessment of hopelessness and despair behavior using forced swimming test (FST)

FST is used frequently to screen for the potential efficacy of prospective anti-depressant agents in rats or mice where the animals are forced to swim in a confined space. A glass cylinder (2.5 cm and 30 cm in height) containing 15 cm water was used. The test was carried out on the last day of the experiment. The measured parameters were immobility, swimming and climbing scores. The duration of immobility reflects the state of behavioral despair. Water was changed before each trial (Parale and Kulkarni, 1986; Porsolt et al., 1977).

2.5. Biochemical assessment

2.5.1. Protein estimation

The protein content was measured in brain homogenates according to Bradford method (Bradford, 1976) using bovine serum albumin as a standard.

2.5.2. Assessment of oxidative stress markers

To assess the oxidative stress, the tissue level of GSH, MDA and SOD were measured in hippocampus homogenate of the different treated groups. GSH was assessed according to the method of Ellman (1959). Also, lipid peroxidation was determined be estimating the level of thiobarbituric acid reactive substances (TBARS) measured as