Toluene Induces Depression-Like Behaviors in Adult Mice

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It has been clinically reported that toluene causes mental depression in humans. However, the detrimental effects of toluene exposure on brain function and the relation between features of mental depression and toluene exposure are poorly understood. This study evaluated depression-like behaviors in adult C57BL/6 mice after administration of toluene, and elucidated the effects of classical antidepressants on the depression-like behaviors. For the estimation of depression-like behaviors, tail suspension test (TST) and forced-swim test (FST) were performed 1, 4 and 16 days after toluene (0~1000 mg/kg bw) treatment. In addition, classical antidepressants such as fluoxetine (FLX, 20 mg/kg bw) and imipramine (IMI, 40 mg/kg bw) were administered 12 h and 1 h before the tests. In the TST and FST, toluene-treated mice exhibited a longer duration of immobility than vehicle-treated mice 1 and 4 days after toluene treatment. The depression-like behaviors were significantly reversed by FLX and IMI. The weight of the adrenal gland and the size of adrenocortical cells were significantly higher in toluene-treated mice compared to vehicle-treated controls. It is suggested that acute toluene exposure of adult mice is sufficiently detrimental to induce depression. In addition, this study has established a mouse model for a depressive state induced by toluene treatment.

Key words: Toluene, Depression, Behavior, Adrenal gland, Animal model

INTRODUCTION

Depression is a serious and incapacitating disorder with a heavy social burden that carries a substantial lifetime risk (Greenberg et al., 2003; Millan, 2004). Severe forms of depression affect 2~5% of the U.S. population, and mood disorders affect 7% of the world’s population and rank among the top 10 causes of disability (Murray and Lopez, 1996). Work-related injuries contribute to the development of psychopathologies such as depression (Stice and Dik, 2009). Work-related depression has emerged as a major cause of long-term sickness. The relationship between work and depression is bidirectional: work gives acceptance and self-confidence to the individual, but workplace stress may precipitate depression (Unger, 2007). Despite its status as the most common psychiatric disorder, depression is poorly understood in terms of the mechanisms.

Toluene is a volatile organic compound that is widely used as a paint-thinner, industrial degreasing agent, and dry-cleaning agent. However, toluene is associated with neuro-physiological and psychological disturbances (Grasso et al., 1984), and it is considered an important health hazard (Fishbein, 1985). Chronic toluene intoxication in humans leads to development of symptoms such as palpitation, insomnia, dizziness with headache, memory impairment, euphoria while working, and mental depression during the weekend relief from work-related toluene exposure (Lee et al., 2003). Similar to those of other sedative-hypnotics, toluene can readily cross the blood-brain barrier and produce central nervous system effects (Balster, 1998). In animal experiments, toluene exposure leads to changes in neurobehavioral and neurobiological functions (Berenguer et al., 2003; Kondo et al., 1995; Reigel and French, 1999; Seo et al., 2010; von Euler et al., 2000). However, little is known about the precise mechanisms of depression in humans and in experimental animal models after toluene exposure.

This study focused on depression-like behaviors using the tail suspension test (TST) and forced swim test (FST) in adult C57BL/6 mice after the administration of toluene. To examine the relationships between toluene-induced depression-like behaviors and stress, the changes of several stress-
related parameters were examined after toluene treatment. Additionally, the effects of antidepressants on toluene-induced depression-like behaviors were evaluated.

**MATERIALS AND METHODS**

**Animals.** Male C57BL/6 mice aged 8–9 weeks (Orient Bio, Gyunggi-do, Korea) were housed in a room that was maintained at 23 ± 2°C, relative humidity of 50 ± 5%, artificial lighting from 08:00–20:00 h and with 13–18 air changes hourly. The animals were given tap water and commercial rodent chow (Samyang Feed, Seoul, Korea) ad libitum. All animal experiments followed a protocol approved by the Committee for Animal Experimentation at Chonnam National University, and the animals were cared for in accordance with the Guidelines for Animal Experiments.

**Toluene administration and tissue sampling.** Toluene (HPLC grade, 99.8% pure) was obtained from Junsei Chemical (Tokyo, Japan). Toluene-treated mice received an intraperitoneal (i.p.) injection of toluene dissolved in corn oil (500 mg/kg bw). Vehicle-treated control mice were injected with only corn oil. I.p. injection of toluene produces the same behavioral symptoms as inhalation (Kondo et al., 1995; Reigel and Frech, 1999) and is easy to handle. In addition, the dosages were selected on the basis of a previous study, which demonstrated the no dosage-related body weight loss and effect on basic locomotor activity (Seo et al., 2010).

The behavior tests were performed at 1, 4 and 16 days after vehicle or toluene administration. To observe the dose-dependent effects of toluene on locomotor activity and depression-like behaviors, mice were administered 0 (vehicle-treated control), 100, 500, or 1000 mg/kg bw dose of toluene, and the behavior tests were performed at 4 days after treatment. In addition, to examine the effect of antidepressants on toluene-induced depression-like behaviors, classical antidepressants, such as imipramine hydrochloride (IMI, 40 mg/kg bw; Sigma-Aldrich, St. Louis, MO, USA) and fluoxetine hydrochloride (FLX, 20 mg/kg bw; Sigma-Aldrich) were dissolved in saline and intraperitoneally administrated 12 h and 1 h, respectively, prior to the behavioral tests.

Body weights of mice were measured 4 days after vehicle and toluene injection, and then the animals were euthanized by ether inhalation. Blood was collected in heparinized tubes and plasma was separated. At dissection, the adrenal gland was also weighed.

For measurement of the cell size of adrenocortical cells, both adrenal glands from each mouse were fixed with 10% neutral-buffered formalin. The tissue sections were stained with hematoxylin and eosin (H&E).

**Open field test.** Open-field analysis was used to measure the activity of the mice in a novel environment. Parameters including ambulatory movement episodes, total moving distance (cm), ambulatory movement time (s), and rest time (s) were determined over 5 min using the TruScan Photo Beam Activity System (Coulbourn Instruments, Whitehall, PA, USA).

**TST.** The TST was similar to that described by Steru et al. (1985). Briefly, mice were suspended from a plastic rod mounted 50 cm above the surface by fastening the tail to the rod with adhesive tape. Immobility was measured for 6 min. Immobility was defined as the absence of any limb or body movements, except those caused by respiration.

**FST.** The FST was similar to that described by Porsolt et al. (1977). Briefly, mice were gently placed in a clear plastic cylinder with a diameter of 13 cm and a height of 23 cm that was filled with 10 cm of clear water at 23–25°C. The test duration was 6 min, and immobility was measured during the last 4 min. Immobility was defined as the absence of any horizontal or vertical movement in the water, but excluded minor movements required for the mouse to keep its head above the surface. The water was replaced before each animal began the test.

**Measurement of the size of adrenocortical cells.** Images of adrenal gland sections were taken with a digital camera mounted on a microscope (Leica DM IRBE; Leica Micro systems GmbH, Wetzlar, Germany). The size of adrenocortical cells in the zona fascicularis was determined using Leica QWin image analyzing software (Leica Microsystems). Two fields (×100) in each sample were measured for the vehicle- and toluene-treated groups.

**Quantitative analysis of serum corticosterone.** The measurement of serum corticosterone was performed 4 days after administration of vehicle or toluene. Blood was collected from vehicle- and toluene-treated mice, and was separated into blood cells and serum. The concentration of serum corticosterone was measured according to the manufacturer’s instructions using a Corticosterone Enzyme Immunoassay kit (Assay Designs, Ann Arbor, MI, USA).

**Statistical analysis.** The data are reported as the mean ± SE and were analyzed using a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls post hoc test for multiple comparisons. In all cases, a p value < 0.05 was considered significant.

**RESULTS**

**Time-related effect of toluene on depression-like behaviors.** TST and FST have been recognized as useful experimental paradigms for assessing depression-like behavior and the activity of antidepressants. These tests were performed at 1, 4 and 16 days after injection of 500 mg/kg bw
Toluene induces depression in mice. Toluene treatment significantly increased immobility time in the TST and FST. These findings support the hypothesis that toluene exposure is associated with depression-like behaviors in mice. Toluene treatment was administered through intraperitoneal injection at doses of 0, 500, and 1000 mg/kg. In the TST, the immobility time significantly increased in toluene-treated mice compared to vehicle-treated controls. Similarly, in the FST, immobility time was significantly increased in toluene-treated mice compared to vehicle controls.

**Effect of antidepressants on toluene-induced depression-like behaviors.** Toluene-induced depression-like behaviors were reversed by the administration of classical antidepressants, imipramine (IMI) and fluoxetine (FLX). Both IMI and FLX significantly decreased the immobility time in toluene-treated mice, indicating their effectiveness in reversing toluene-induced depression-like behaviors.

**Effect of toluene in the locomotion in the open-field test.** Basic locomotor activity of mice was measured in an open-field test after toluene treatment. Toluene exposure decreased locomotor activity, as evidenced by a decrease in ambulatory movement count and an increase in time spent immobile. This effect was dose-dependent, with the highest decrease observed at the highest dose of toluene.

**Dose-related effect of toluene on depression-like behaviors.** TST and FST were performed after toluene injection to examine dose-related depression-like behaviors. As shown in Fig. 2A, the TST-determined immobility time increased progressively with increasing dose of injected toluene (0–1000 mg/kg), although there was no significant difference up to 100 mg/kg toluene. In FST, the immobility time significantly increased at 100, 500, and 1000 mg/kg of toluene compared to vehicle controls (0 mg/kg), but leveled off slowly as the dose was increased (Fig. 2B).

**Fig. 1.** Time-dependent effect of toluene injection on immobility measured during the TST (A) and FST (B) in adult mice. Vehicle (corn oil) or toluene (500 mg/kg bw) was injected at 1 day, 4 days and 16 days before the test. (A) Immobility time in the TST significantly increased in toluene-treated mice compared to vehicle controls. (B) Similarly, immobility time in the FST significantly increased in toluene-treated mice compared to vehicle controls. However, mice examined 16 days after toluene injection showed no significant difference in immobility. (B) In the FST, the immobility time in mice was significantly higher in toluene-treated mice than in vehicle-treated controls, but not in mice 16 days after toluene injection, similar to the results of TST. The values reported are the mean ± SE (n = 7 per group). *p < 0.05, **p < 0.01, ***p < 0.001 vs. vehicle-treated controls.

**Fig. 2.** Dose-dependent effect of toluene injection on immobility measured during the TST (A) and FST (B) in adult mice. Vehicle (corn oil) or toluene (100, 500 and 1000 mg/kg bw) was injected 4 days before the test. (A) In the TST, mice injected 100 mg/kg bw toluene showed similar time spent to immobile (s) with vehicle controls. However, mice injected 500 and 1000 mg/kg bw toluene showed significantly higher time spent to immobile than vehicle-treated control mice. (B) In the FST, mice injected toluene in all of selected doses showed higher time to spent immobile than vehicle-treated control mice. The values (seconds) reported are the mean ± SE (n = 5 per group). **p < 0.01, ***p < 0.001 vs. vehicle-treated controls.
depression-like behavior. However, the effects of the two antidepressants differed, in that IMI strongly decreased the immobility time, whereas FLX produced only a marginally significant decrease (IMI: 81.5 ± 24.7 sec, \( p < 0.001 \); FLX: 190.2 ± 4.29 sec, \( p < 0.05 \) vs. toluene-treated group: 207.0 ± 4.33 sec, \( n = 6 \) ) in contrast to the effects in TST (Fig. 3B).

**Effect of toluene on stress-related parameters.** Total adrenal gland weight per body weight, cortical cell size and concentration of corticosterone were examined as stress-related parameters to evaluate the effect of toluene (0–1000 mg/kg bw) injection (Table 2). All the doses of toluene significantly induced increased adrenal weight per body weight (\( \mu g/mg \)) (100 mg/kg bw: 242.3 ± 8.88, \( p < 0.05 \); 500 mg/kg bw: 234.7 ± 8.86, \( p < 0.05 \); 1000 mg/kg bw: 256.0 ± 9.39, \( p < 0.01 \) vs. vehicle-treated control: 206.6 ± 8.43, \( n = 9 \) ) 4 days after injection compared to vehicle-treated controls. Similarly, cortical cell size was also significantly increased by all selected doses (100 mg/kg bw: 52.1 ± 2.27 \( \times 10^2 \) \( \mu m^2 \), \( p < 0.01 \); 500 mg/kg bw: 50.0 ± 3.02 \( \times 10^2 \) \( \mu m^2 \), \( p < 0.05 \); 1000 mg/kg bw: 57.0 ± 2.19 \( \times 10^2 \) \( \mu m^2 \), \( p < 0.0001 \) vs. vehicle-treated control, \( n = 9 \) ) 4 days after injection compared to vehicle-treated controls. Furthermore, the changes in concentration of serum corticosterone were more pronounced in cells treated with all doses of toluene, as compared to vehicle-treated control mice 4 days after injection, although the changes were not statistically significant (Table 2).

## DISCUSSION

Presently, toluene exposure induced depression-like behaviors as measured by TST and FST in adult mice. In addition, these depression-like behaviors induced toluene exposure reverse by two classical antidepressants. This suggests that toluene exposure causes depression in adult mice.

Long-term recreational, occupational, as well as environmental exposure to toluene may result in a variety of neurological manifestations, including ataxia, dementia, sensory

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### Table 1. Open-field analysis of mice placed in a novel environment at 4 days after vehicle (0 mg/kg bw) and toluene (100–1000 mg/kg bw) injection

<table>
<thead>
<tr>
<th>Toluene dose (mg/kg bw)</th>
<th>0</th>
<th>100</th>
<th>500</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Movement episodes</td>
<td>29.2 ± 2.67</td>
<td>27.0 ± 2.85</td>
<td>25.4 ± 2.68</td>
<td>33.6 ± 5.26</td>
</tr>
<tr>
<td>Distance (cm)</td>
<td>448.1 ± 88.6</td>
<td>466.8 ± 10.6</td>
<td>470.6 ± 28.0</td>
<td>387.2 ± 35.1</td>
</tr>
<tr>
<td>Movement time (sec)</td>
<td>225.8 ± 5.66</td>
<td>233.4 ± 3.31</td>
<td>236.2 ± 3.40</td>
<td>211.0 ± 9.29</td>
</tr>
<tr>
<td>Rest time (sec)</td>
<td>38.8 ± 2.48</td>
<td>36.0 ± 4.49</td>
<td>30.4 ± 3.87</td>
<td>44.4 ± 6.64</td>
</tr>
</tbody>
</table>

The data are reported as the mean ± SE (\( n = 5 \) per group).

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### Table 2. Changes of the examined markers between vehicle-treated control group (0 mg/kg bw) and toluene-treated group (100–1000 mg/kg bw) at 4 days after injection (% of control)

<table>
<thead>
<tr>
<th>Toluene dose (mg/kg bw)</th>
<th>0</th>
<th>100</th>
<th>500</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal weight per body weight</td>
<td>100 ± 4.08</td>
<td>117.2 ± 4.30*</td>
<td>113.6 ± 4.29*</td>
<td>123.9 ± 4.54**</td>
</tr>
<tr>
<td>Cortical cell size</td>
<td>100 ± 4.19</td>
<td>123.7 ± 4.92**</td>
<td>116.3 ± 5.08*</td>
<td>135.5 ± 4.36***</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>100 ± 31.60</td>
<td>146.4 ± 24.39</td>
<td>139.1 ± 21.14</td>
<td>143.1 ± 33.30</td>
</tr>
</tbody>
</table>

The data are reported as the mean ± SE (\( n = 9 \) per group). * \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \) vs. vehicle-treated controls.
Toluene Induces Depression

Dysfunction, seizure, tremor and cognitive impairment, as well as depression (Benignus, 1981; Anderson and Loomis, 2003). Recently, Crez et al. (2009) reported on the antidepressant-like actions of toluene 30 min after inhalation. In contrast, another study reported that hippocampal dysfunctions such as cognitive impairment and depression were evident up to 4 days following toluene exposure (Seo et al., 2010). The present data confirmed that toluene exposure induces depression-like behaviors in the short-term (1 and 4 days) reversibly, but not in the long-term (16 days) after acute toluene exposure in mice. This may reflect a time-dependent effect of toluene exposure.

Depression is a highly ubiquitous, complex and heterogeneous disorder with serious physical, mental and socioeconomical consequences. Among the mechanisms associated with depression, the role of stress in psychiatric disorders has been well-demonstrated; in particular, epidemiological data have lent strong support to the idea that stressful life events play a role in the etiology of depression (Kendler et al., 1995). However, the precise mechanisms of the depression-like behaviors induced by toluene remain unknown. Here, two possible mechanisms can be offered. One theory posits that the genesis of depression is reduced brain plasticity. According to this theory, depression may be due not only to the changes in neurotransmitter concentrations and receptor activity levels, but also to impaired brain plasticity and tissue remodeling, and alterations in adult hippocampal neurogenesis (Kim et al., 2008). The hippocampus is one of several limbic structures that have been extensively studied in individuals with learning and memory difficulties, and depression (Kim et al., 2008; Seo et al., 2010; Yang et al., 2010). Toluene exposure in adult mice may reduce hippocampal neurogenesis and causes hippocampal dysfunctions such as depression and cognitive impairment (Seo et al., 2010). Therefore, this reduction of neurogenesis by toluene exposure alters brain plasticity, and may induce hippocampal dysfunction, including depression.

Another mechanism is stress-related depression. Toluene exposure induces adrenocortical hypertrophy via the stress-responsive hypothalamus-pituitary-adrenal gland (HPA) axis, neither stimulating nor damaging adrenal cells directly (Gotohda et al., 2005). As previously reported, the present study observed the significant enlargement of the adrenal gland, especially adrenocortical cells, upon toluene exposure. Additionally, the concentration of corticosterone was elevated, albeit non-significantly. Also, toluene induces the impairment of energy metabolism, oxidative stress in the prefrontal cortex and hippocampus-related chronic stress (Tagliari et al., 2010). Therefore, toluene may induce depression via direct facilitation of the corticosterone excretion, or indirect damage to hippocampus such as impaired energy metabolism and oxidative stress. However, further studies should clarify the molecular and cellular mechanisms for the in vivo depression effects of toluene exposure.

In conclusion, toluene exposure transiently induces depression-like behaviors in TST and FST, and these behavioral effects are reversed by antidepressants, suggesting that toluene exposure induces depression in adult C57BL/6 mice. Additionally, the behavioral effects are related with several stress-related parameters, such as weight of adrenal glands, size of adrenocortical cells and concentration of corticosterone. This study has established a mouse model of a depressive state induced by toluene treatment. This suggests that acute toluene treatment in mice is a beneficial animal model for screening new antidepressants and clarifying the precise mechanisms of mental depression.

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