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The Effect of Idesolide on Hippocampus-dependent Recognition Memory

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Abstract: Finding a way to strengthen human cognitive functions, such as learning and memory, has been of great concern since the moment people realized that these functions can be affected and even altered by certain chemicals. Since then, plenty of endeavors have been made to look for safe ways of improving cognitive performances without adverse side-effects. Unfortunately, most of these efforts have turned out to be unsuccessful until now. In this study, we examine the effect of a natural compound, idesolide, on hippocampus-dependent recognition memory. We demonstrate that idesolide is effective in the enhancement of recognition memory, as measured by a novel object recognition task. Thus, idesolide might serve as a novel therapeutic medication for the treatment of memory-related brain anomalies such as mild cognitive impairment (MCI) and Alzheimer's disease.

Key words: idesolide, recognition memory, novel object recognition task

In recent years, there have been many attempts to enhance human cognitive functions and to cure mild cognitive impairment (MCI), which is a common attribute of ageing and occasionally leads to the more serious mental disorder, dementia.

Up to now, most of these efforts turned out to be futile and MCI is still untreatable, because none of the medications developed for it have been effective in improving human cognitive functions, and a good number of them had too many side effects to be tolerated. Nevertheless, further endeavors to alleviate MCI, and to search for more efficacious drugs with fewer side effects, have been started, because of the fact that MCI is directly associated with poor quality of life of many people and results in vast socioeconomic costs.

In an effort to improve human cognitive functions without serious side effects, some studies have begun to focus on the effects of herbal extracts on neurons, synaptic functions and the brain. In the case of natural products, reports of unfavorable side effects are limited and positive effects on the brain, such as neuroprotection and recovery of memory impairment, have been reported (Lee et al., 2006, Koo et al., 2006, Kim et al., 1998). For example, herbal medicines such as *Ginko biboba*, Ginseng or *Melisa officinalis*, have been described to have cognition- and memory-enhancing effects (Birks et al., 2002, Kennedy et al., 2003). In addition, extracts from *Angelica gigas* and *Callicarpa dichotoma* brought about relief of memory impairment caused by scopolamine, a muscarinic antagonist known to have detrimental effects on learning and memory in rodents and humans (Lee et al., 2006, Kang et al., 2003, Beatty et al., 1986, Collerton, et al., 1986, Kopelman et al., 1988). In a similar manner, several compounds from natural products were found to have neuroprotective effects in a primary rat cortical culture system (Koo et al., 2006, Kim et al., 1998).

In this study, we examine the effect of a herbal extract idesolide on hippocampus-dependent recognition memory. Idesolide, which is isolated from the fruits of *Idesia polycarpa* Maxim, is a spiro compound possessing a tetrahydrobenzodioxole structure (Kim et al., 2005). A previous study reported that idesolide rendered inhibitory activity against lipopolysaccharide(LPS)-induced nitric oxide production in BV2 microglial cells, leading to antiinflammation (Kim et al., 2005). Here we hypothesize that idesolide might also be capable of improving hippocampus-dependent recognition memory in rodents and this possibility is probed throughout the study.

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MATERIALS AND METHODS

Animals

All the behavioral experiments were carried out on male C57BL/6J mice. Mice were kept on a 12 h light/ 12 h dark cycle, and the behavioral experiment was performed during the light phase of the cycle. Food and water were always provided *ad libitum* and only 9 week old male mice were used throughout the experiments.

Novel object recognition task

A novel object recognition task was performed in an open field made from opaque acryl. On the day of the sample phase, idesolide (40 μg/kg) was injected IP 30 min before training. Mice were placed on the open field for 15 min with two identical objects. On the retention phase test, which was performed 24 h after the sample phase, mice were placed on the open field again for 10 min with two different objects (one was the same object as that presented at the sample phase, the other was a new object only presented at the retention phase). The time spent exploring each object was recorded. The discrimination index was calculated as the difference in time (AT-BT) spent by each animal exploring the new object compared with the familiar object divided by the total time (AT+BT) spent exploring both objects. Object A was an electric bulb and object B was a glass vial. Preference was derived from the exploration time for the novel object divided by the total time spent exploring both objects.

Data analysis

An unpaired *t*-test was used to analyze the novel object recognition task data. Significance was established at $p< 0.05$. All data were expressed as a mean±the standard error of the mean (SEM). Experimenters were blind to each experimental group following behavioral tests.

Idesolide

The fruits of *Idesia polycarpa* were collected in Beagwoon Mountain, Gwangyang city, Jeollanam-do, Korea. Fresh fruit (9 kg) was ground and extracted with MeOH (3×10 L) at room temperature. The MeOH extract was concentrated in vacuo to give a crude extract (1.2 kg) and then partitioned successively with *n*-hexane, $CHCl_3$, ethylacetate and *n*-BuOH. The $CHCl_3$ fraction (32 g), which showed the most potent anti-inflammatory activity, was subjected to ODS gel column chromatography with a gradient elution of H_2OMeOH to give nine fractions (CI to CIX). CII was applied on a silica gel column using a mixture of $CHCl_3$-MeOH as an eluting solvent to give two subfractions (CII-1 to CII-2). Idesolide (130 mg) was isolated from CII-1 by semi-preparative HPLC (Microsorb C_{18} 80-299, 10×250 mm, H_2O : MeOH = 80 : 20, 20 ml/min, t_r = 25.8 min) (Kim et al., 2005).

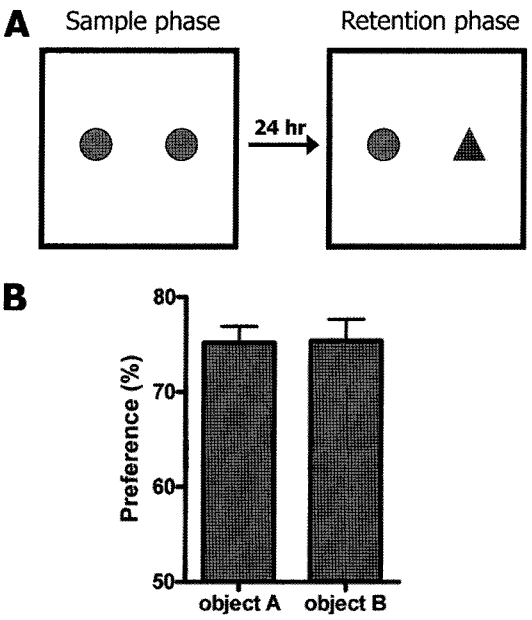

Fig. 1. Novel object recognition task and preference test. (A) The illustration describes a novel object recognition task protocol consisting of two phases (sample phase and retention phase). In the sample phase, two identical objects are presented to the mice and in the retention phase, one of the two objects is replaced by a novel one. (B) The preference level for the two different objects used in our experiment is almost the same, which indicates that mice have no innate predisposition to either object in the novel object recognition task (object A; n = 8, object B; n = 9 per each group). Error bars indicate SEM.

RESULTS

Novel object recognition task

The novel object recognition task has been widely used to measure hippocampus-dependent recognition memory in rodents (Mumby, 2001, Broadbent et al., 2004, Clark et al., 2000). The task consists of two phases (the sample phase and retention phase). In the sample phase, mice are given a chance to explore two identical objects in the open field. Twenty four hours later, in the retention phase, mice are exposed to two different objects again, one familiar and the other novel (Fig 1A). Typically, mice prefer to explore the novel object and the level of preference to the novel object is measured and perceived as an indicator of memory extent.

Before the experiments, we first tried to confirm that the mice we used had no inborn preference at all between the two types of objects (a vial and a bulb). The results from the preference test showed that mice had no natural preference for either of the objects (object A (vial), 75.2±1.7%; n = 9 vs. object B (bulb), 75.4±2.6%; n = 8; unpaired t-test, $P> 0.05$) (Fig. 1B). Therefore, we concluded that these two different objects were suitable for the novel object recognition task.

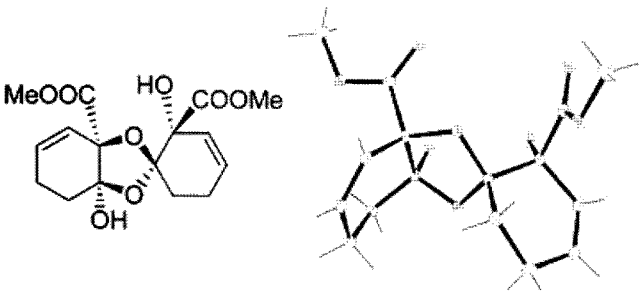

Fig. 2. Chemical and X-ray structures of idesolide. Idesolide is a spiro compound possessing a tetrahydrobenzodioxole structure (left). Its X-ray structure was revealed by NMR and MS spectroscopic analysis (right) (Kim et al., 2005).

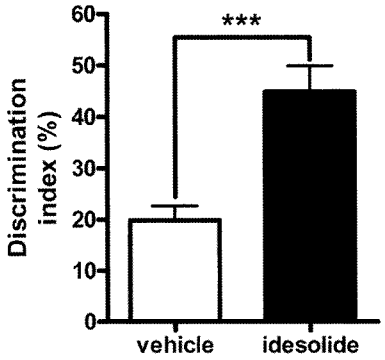

Fig. 3. Idesolide is effective in enhancing recognition memory tested for novel object recognition task. At an idesolide concentration of 40 ig/kg, the mice group injected with idesolide (n = 10) showed a significantly higher level of discrimination index compared with the mice injected with vehicle (n = 10) in the novel object recognition task. Error bars indicate SEM. *** indicates *p*-value < 0.001.

Idesolide-its effect on enhancement of recognition memory

Next we examined whether idesolide, a new compound extracted from *Idesia polycarpa,* had an effect on cognitive memory enhancement. *Idesia policarpa* is a deciduous tree of the Flacourtiaceae family and is native to some Asian countries including Korea and China (Kim et al., 2005). Idesolide is a new spiro compound having tetrahydro-benzodioxole structure. Its structure was established by NMR and MS spectroscopic analysis with single-crystal X-ray experiments (Fig. 2A) (Kim et al., 2005). Idesolide has been known to have an anti-inflammatory effect; the inhibitory activity of this compound against LPS-induced nitric oxide production was demonstrated in BV2 microglial cells (Kim et al., 2005). Based on these reports, we tested a potential cognitive memory enhancement effect by idesolide using the novel object recognition task. By injecting idesolide intraperitoneally (40 μg/kg) 30 min before the sample phase, the effect of idesolide on the acquisition state of memory could be evaluated. Twenty four hours after the sample phase test, in which two identical objects were exposed to mice for 15 min in an open field, the retention phase test was performed. During the test two different objects were brought to the mice for 10 min in the same open field again; one object was the same as that presented in the sample phase, but the other was novel to the mice. One of the two objects was presented to the mice as a novel one in a counterbalanced manner. During the retention phase test the time spent exploring the two different objects was recorded individually. The outcome revealed that mice in the group treated with idesolide had a significantly higher level of recognition memory in the retention phase compared with the vehicle-injected group (vehicle, 19.8 ±2.8% vs. idesolide; 40 μg/kg, 49.1±6.1%; unpaired t-test, $P<0.001$) (Fig. 3). These data suggested that the intraperitoneal injection of idesolide in the sample phase might influence acquisition of recognition memory and that this effect was manifested in the form of increased exploration time to the novel object. In conclusion, this result shows that idesolide, while having an anti-inflammatory effect on microglial cells, also has a therapeutic potential to enhance cognitive memory functions in rodents.

DISCUSSION

In this study, the effect of idesolide, which was extracted from *Idesia polycarpa,* on hippocampus-dependent recognition memory was examined using the novel object recognition task paradigm. Compounds extracted from medicinal herbs or other natural sources have been widely investigated and known to retain a variety of potent biological functions. Moreover, a great body of evidence from animal and human studies has demonstrated that cognitive functions such as learning and memory could be altered by herbal medicines or their extracts (Kennedy et al., 2003, Wei et al., 2000, Lee et al., 2006, Kang et al., 2003). In the similar vein, our result shows that idesolide treatment caused enhancement of hippocampus-dependent recognition memory, which was represented by the increment of exploration time in mice towards a novel object. The detailed mechanism underlying the memory enhancing effect by idesolide was not explored in our study. However, previous researches provide some clues about a plausible mechanism of idesolide effect on memory enhancement. Koo et al. (2006) showed that compounds isolated from both *Biota orientalis* and *Callicarpa dichotoma* leaves rendered neuroprotective activity against glutamate-induced toxicity in primary cultured rat cortical neurons. In another study, the aqueous extract of the herbal formula Liuweidihuang-tang increased neurogenesis in the dentate gyrus of hippocampus in rats (Lee et al., 2005). Also, sauchinone, a lignan from *Saururus chinensis,* inhibited staurosporine-induced apoptosis in C6 rat glioma cells (Song et al., 2003).

In the case of idesolide, its anti-inflammatory effect has been studied and it was shown that idesolide treatment suppressed the activity of microglial cells, which played an important role in the brain immune system (Kim et al., 2005). Earlier studies demonstrated that anti-inflammatory activity could be related to neurogenesis or the survival of newborn neurons (Lim et al., 2005, Hoehn et al., 2005). Neurogenesis in rats after focal cerebral ischemia was enhanced by indomethacin, a nonsteroidal drug that had an anti-inflammatory action, and indomethacin reduced microglial activation (Hoehn et al., 2005). On the other hand, recent studies indicate that neurogenesis in the hippocampus may be related to hippocampus-dependent learning and play a pivotal role in the formation of some types of memory (Gould et al., 1999, Shors et al., 2001, Shors et al., 2002, Dalla et al., 2007). Furthermore, various neuro-inflammatory mechanisms are often associated with neurodegenerative diseases such as Alzheimer's disease (Heneka et al., 2007). If this is the case, the injection of idesolide may have an impact on the brain immune system, and its anti-inflammatory activity might enhance neurogenesis or provide a potent neuroprotective effect on glutamate-induced neurotoxicity, leading to the elevation of cognitive functions. While the detailed mechanisms of the effect of idesolide on cognitive functions remain to be elucidated, the present study suggests that idesolide may be utilized as a novel memory-enhancing drug without adverse side effects and the anti-inflammatory action of idesolide on the neural immune system might underlie its therapeutic potency.

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