Effects of Edaravone on Scopolamine Induced-dementia in Experimental Rats

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Abstract: It has been shown that edaravone and scopolamine have contrasting effects on memory; therefore, this research paper was undertaken to evaluate the impact of edaravone treatment on learning and long-term memory shortage coupled with scopolamine induced dementia. The results showed that chronic edaravone treatment averted the deficit of long-term memory as measured by transfer latency using spatial cues in the elevated plus maze task. Moreover, edaravone protected against the weakening of antioxidant defense activity in the areas of hippocampi and cerebral cortices of scopolamine treated rats. Furthermore, the thiobarbituric acid reactive substances test revealed that edaravone prevented the detrimental effects of scopolamine on lipid peroxidation (p<0.01). The results suggest that edaravone treatment protected against the scopolamine generated memory deficit probably by preserving the levels of reduced glutathione and TBARS. Hence, it is found to possess neuroprotective effects in scopolamine induced memory impairment model.

Key words: Edaravone, scopolamine, memory impairment, neuroprotective effects

INTRODUCTION

The oxidative stress is one of most important factors for the neurodegeneration of brain leading to various disorders (Facchinetti et al., 1998; Southorn and Powis, 1998; Halliwell, 2001). Additionally, oxidative damage is considered as prevailing factor for aging and cells tend to be exposed to free radicals, which in turn lead to permanent damage of brain cells and neuronal death. Currently, Alzheimer's disease accounts for large number of prevalence, morbidity and mortality in United States and it is estimated that one in every three aged adults are dying with Alzheimer's disease (Kehrer, 1993; Southorn and Powis, 1998; McCord, 2000; Devasagayam et al., 2004). Free radicals generated within the brain are the major factor in Alzheimer's disease (Ikeda and Long, 1990). Reactive oxygen species causes permanent irreversible oxidative damage to brain cells, which further aggregates to loss of memory or dementia (Stohs, 1995; Valko et al., 2007).

The reduced glutathione and lipid peroxidation are well known elements to play an essential role in oxidative stress balance. The reduced glutathione represents the endogenous defense against oxidative stress, while lipid peroxidation illustrates the damage into the cells. Therefore, the decrease in the activity of reduced glutathione and enhancement in the thiobarbituric acid reactive substances may lead to oxidative stress (Freeman and Crapo, 1982; Halliwell, 1992; Reiter, 1995).

Scopolamine, a muscarinic receptor blocker, has been used to assess amnesia in different animals (El-Sherbiny et al., 2003). It causes memory impairment by anti-cholinergic actions which is an useful model to check the anti-amnesic effects of drugs (El-Sherbiny et al., 2003; Mishima et al., 2003). There is considerable evidence that scopolamine causes oxidative stress in rats leading to cognitive impairment (El-Sherbiny et al., 2003; Mishima et al., 2003). Taken together, scopolamine leads to oxidative stress through the interference with acetylcholine in central nervous system (Shi et al., 2010). Hence, we have selected this model as one of most suitable method to study dementia (Kang et al., 2003). It is understood from the fact the free radicals increases oxidative stress in brain leading to memory impairment. However, its precise mechanism is still not clear.

Edaravone is a free radical scavenger and several reports have shown that there is inverse correlation between edaravone administration and oxidative stress (Otomo et al., 2003; Zhou et al., 2013). In fact, investigators have reported that edaravone produces beneficial effects on oxidative stress markers, including glutathione, superoxide dismutase, malonaldehyde and glutathione peroxidase. Therefore, the use of edaravone as a neuroprotective agent was suggested as a therapeutic drug for recovery of neurological disorders and treatment of neurodegenerative disorders (Otomo et al., 2003; Yoshida et al., 2011; Kikuchi et al., 2012; Zhou et al., 2013). However, the mechanism by
which edaravone antagonizes the sequence of free radical
cycle and ameliorates the uncontrollable and irreversible
oxidative state is under development. On the basis of
literature, our study was to investigate the action of
edaravone on spatial learning and memory deficit
associated with scopolamine.

MATERIALS AND METHODS

Animals: Adult male albino rats were distributed
randomly and a set of six animals were housed together.
The cages were in animal facility with a temperature of
about 24°C. All rats were allowed to acclimate for one
week before the beginning of treatment. Three
experimental classes were assigned; control, scopolamine,
scopolamine and edaravone before the acclimation of
seven days.

Drugs and chemicals: Edaravone and scopolamine were
purchased from M/s. Sigma Chemicals, India.

Preparation of dosage form: Edaravone (15 mg kg⁻¹
orally) was dissolved in DMSO and administered for
seven days. The DMSO was used as solvent for
scopolamine (1mg kg⁻¹), which was administered
intraperitoneally on 7th day of the experiments.

Experimental protocol

- Group 1: Received vehicle (Saline with CMC) for
  seven consecutive days
- Group 2: Scopolamine hydrobromide 1 mg kg⁻¹
  intraperitoneal at 7th day
- Group 3: Alzheimer induced+(Edaravone 15 mg kg⁻¹
  for seven days)

Elevated plus maze model: The cognitive function of all
three groups were assessed through the elevated plus
maze task. Several lines of evidence have shown that
elevated plus-maze model can be used to test the
hippocampus-dependent memory using spatial cues. The
elevated plus-maze technique was composed of a central
platform linked into four metal pieces, which form plus
sign shape. The diameter was 50×10 cm for the opened
metal and 50×40×10 cm for the closed one. The height of
central platform and four limbs was 50 cm from the ground
(Itoh et al., 1990). During the learning phase (i.e., seven
days after the beginning of edaravone administration),
each rat was located in one of the sides which was
opened. Transfer latency was considered as the required
time (in seconds) by which the rat moves from the open
limb until it reaches any one of the closed limbs. Transfer
latency was documented during the learning phase for
each rat on day 7th. Twenty four hours later, the long-
term memory test was performed (on the 8th day). The
decrease and increase in the transfer latency indicate
memory improvement and impairment, respectively.

Lipid peroxidation assay: In the current study, the
thiobarbituric acid reactive substance was used to as an
indicator find out lipid peroxidation (Ohkawa et al., 1979;
Mattson, 2009). The supernatant of homogenate was
added to 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL
of 30% acetic acid (pH 3.5), 1.5 mL of 0.8% of
thiobarbituric acid in a test tube. Then after, The test
vials were kept for one hour at 95°C, then 1 mL of
distilled water was added, subsequently 5 mL of
n-butanol-pyridine mixture (15:1 v/v) was added. The
mixture was then centrifuged at 4000 g for 10 min. After
that, the absorbance was determined at 532 nm using
spectrophotometer.

Reduced glutathione level: The level of reduced
 glutathione within the hippocampus and cerebral cortex
was calculated based on technique described by
Beutler et al. (1963). In brief, the combination of
trichloroacetic acid (10% w/v) and supernatant of
homogenate in a ratio of 1:1 was centrifuged at 1000 g at
40°C. Then, 0.5 ml of supernatant has been added into 2
mL of 0.3 M disodium hydrogen phosphate and 0.25 mL
of 0.001 M freshly prepared [5, 5'-dithiothiobis(2-nitrobenzoic
acid) dissolved in 1% w/v citric acid]. The absorbance
was measured at 412 nm through spectrophotometer.

RESULTS

Elevated plus maze studies: Edaravone averts learning
and long-term memory deficit associated with scopolamine induced dementia.

In this experiment, we examined the impact of
edaravone on scopolamine-induced spatial learning and
long-term memory impairment using the elevated plus
maze method. Transfer latency on seventh day of
edaravone represented the learning acquisition, while
transfer latency of next day (eighth day) indicates the
long-term memory performance.

In spatial learning test, administered 45 min at day
7th, the scopolamine treated group committed
significantly (p<0.01) more time (seconds) in finding the
closed arm in the elevated plus maze than the control
group (control: 22.87±4.41; scopolamine: 61.00±9.16)
(Fig. 1a) indicating marked impairment of learning. Chronic
edaravone treatment prevented the increase in the number
of seconds in the scopolamine group (18.21±6.12) as
Antioxidant activity: To reveal the alterations in the levels of oxidative stress-related molecules that may account for the ability of edaravone to avert memory impairment in scopolamine-induced cognitive loss, we determined the levels of reduced glutathione and thiobarbituric acid reactive substances from the tissue homogenate of hippocampus and cerebral cortex.

Effect of edaravone on oxidative damage marker: The Thiobarbituric Acid Reactive Substances (TBARS) is widely considered as the most common assay to measure the lipid peroxidation. In the current study, the scopolamine group revealed an increase in the TBARS levels compared to control group. In edaravone-treated rats, chronic treatment of edaravone for seven days prevented the increased in the levels of TBARS (p<0.001) as a result of single i.p. injection of scopolamine, but did not restore it as in control rats (p<0.01) (Table 1).

Effect of Edaravone on Reduced glutathione levels: Reduced glutathione is an indicator for the ability of the tissues to neutralize the free radicals (Table 2), the scopolamine treated rats showed that the levels of reduced glutathione were markedly (p<0.01) decreased (6.93±0.03) compared to the control rats (10.11±0.03). However, chronic i.p administration of edaravone prevented the effects of scopolamine on the reduced glutathione, which was significantly (p<0.001) increased the edaravone treated rats (10.76±0.018).

DISCUSSION

Various reports have shown that edaravone and scopolamine produce beneficial and detrimental actions.
on cognitive function, respectively. However, the collective impacts of edaravone and scopolamine on cognition have not been studied. I assessed the impact of edaravone administration on memory deficit produced by scopolamine through two procedures, behavioral and molecular. The findings suggest that administration of edaravone for seven days prevents scopolamine-induced long-term memory through normalizing the balance between reduced glutathione and lipid peroxidation.

There is growing body of evidence in the literature that indicates oxidative stress as crucial factor for several disorders including, dementia. Furthermore, data indicates that oxidative stress is one of the earliest events in pathogenesis of memory impairment (Facchinetti et al., 1998; Southorn and Powis, 1998; Halliwell, 2001; Butterfield et al., 2002; Devasagayam et al., 2004; Silva et al., 2004; Suzuki et al., 2006). The cognitive deficit in several diseases demonstrates oxidative damage via inequity on the oxidative stress balance Castellani et al., 2001; Halliwell, 2001; Bradley et al., 2010).

Scopolamine has been commonly used to induce memory deficits in experimental rats through antagonizing muscarinic cholinergic receptors. Several reports have shown that single intraperitoneal (i.p.) injection of scopolamine blocks cholinergic transmission and impairs cognition in rats (Fan et al., 2005; Alikatte et al., 2012). In consistent with that our findings show that an i.p. dose of scopolamine damage learning and long-term memory as indicated by the increase in the transfer latency compared to control rats. Lately, it has been found that the decline in cognition as a result of single injection of scopolamine has been accompanied with changes in the brain oxidation condition (Wang et al., 2014). In our study, the markers of oxidative stress were measured to investigate the mechanism underlies memory impairment in scopolamine group. The results suggest that the memory deficit was as a result of an enhancement of lipid peroxidation and reduction in reduced glutathione within the areas of hippocampus and cerebral cortex.

Edaravone, a chemical with antioxidant effect, has been studied to explore its sequences at the behavioral level. The edaravone prevents long-term memory impairment induced by Alzheimer's disease using Morris water maze procedure (Yoshida et al., 2006; Kamida et al., 2009; Zhou et al., 2013). In the present experiments, the elevated plus maze was used to evaluate the action of edaravone on memory deficit associated with scopolamine. Edaravone protected against the decrease in the transfer latency in scopolamine group. Similar result was reported by Ueno in Japan which showed that protective action after 3 day treatment in rat chronic hypoperfusion model (Ueno et al., 2009). A study on Indian patients showed significant improvement after two weeks of treatment with edaravone. Furthermore and found to be harmless and efficient in treatment of neurodegenerative disorders (Sinha et al., 2009).

Various reports have suggested that edaravone inhibits vascular endothelial damage and hinders neurodegeneration. Indeed, pretreatment of edaravone has been found to reduce the apoptosis in hypoxic-ischemic rats (Yasukoa et al., 2004). Furthermore, several lines of evidence have demonstrated the positive effects of edaravone on lipid peroxidation and antioxidant enzymes. The use of edaravone has been found to decrease hydroxyl radicals and superoxide anions levels (Pan et al., 2010). The current findings in hippocampus and cerebral cortex support the role of edaravone on oxidative stress. The current study shows that edaravone enhanced the activity of reduced glutathione and reduced the activity of TBARS. Therefore, the protective effects of edaravone on spatial memory impairment as demonstrated by elevated plus maze could be as a result of the antioxidant activity.

REFERENCES


