Effect of flumazenil on memory retrieval determined by trial-to-criteria inhibitory avoidance method in mice

Sir,

In India, the incidence of Alzheimer's disease (AD) is substantial and is approximately 14 times higher among persons older than 85 years compared with those between 65 and 69 years of age.[1] In a study conducted in Southern India, incidence of AD was found to be 11.67% in 55 years and above age group and 15.54% in 65 years and above age group.[2] Currently approved drugs in the treatment of AD act on cholinergic system (e.g. donepezil) and glutamatergic system (e.g., memantine).[3,4] Treatment of AD till date is far from satisfactory. Recent failures of several phase III trials of many potential drug molecules for the treatment of AD have reiterated the need to find new molecular targets.[5] In addition, the role of other neurological mechanisms, for example, gamma-aminobutyric acid (GABA)-ergic system as a potential target for the treatment of AD has not been explored in details.

Benzodiazepines are the drugs that selectively bind to modulatory site of $GABA_A$ receptor.[6] This modulatory site is different from GABA binding site and is specific for benzodiazepines. The discovery of specific benzodiazepine receptors in the central nervous system of higher vertebrates led to subsequent identification of possible endogenous effectors (benzodiazepine endocoids or endozepines) for these receptors.[7,8] A study done by Kumar *et al*. has shown that CGS 8216, a benzodiazepine receptor antagonist, enhances learning and memory in mice by inhibiting the action of endozepines.[9]

Flumazenil is a benzodiazepine receptor antagonist. It is used to reverse the adverse psychomotor, amnestic, and sedative effects of benzodiazepine receptor agonists.[10] Flumazenil has also been found to accelerate acquisition, improve retention, and protect against experimental amnesia in mice.[11] In AD, the defect in memory retrieval is associated with long-term memory loss.[1] Previously, effects of flumazenil in memory acquisition have been studied,[11] but its effect on memory retrieval is yet to be explored. Hence, this study was planned to evaluate the effect of flumazenil on memory retrieval in mice.

The study was conducted in two parts. In part 1, the effect of flumazenil 40 mg/kg[11] on learning and memory performance in normal Swiss albino mice was studied in trial-to-criteria inhibitory avoidance.[12] In part 2, flumazenil 40 mg/kg[11] was tested for its effect on learning and memory performance in mice with scopolamine-induced amnesia in trial-to-criteria inhibitory avoidance. Permission of the Institutional Animal Ethics Committee of Seth Gordhandas Sunderdas Medical College and KEM Hospital, Mumbai, was taken before commencement of the study (AEC/02/12). The study was conducted in accordance with the Committee for the purpose of Control and Supervision of Experiments on Animals guidelines. The data were expressed as mean ± standard deviation for each group. An unpaired *t*-test was performed to compare latency to step into the dark area between the two study groups. The level of significance was at $P < 0.05$.

When the effect of flumazenil on memory retrieval in normal animals was tested, the transfer latency in flumazenil group (131.75 ± 62.62) was similar to that of vehicle control (119.25 ± 61.82).

When scopolamine was administered 5 min prior to the test session; there was a decrease in transfer latency time.

The reduction in transfer latency was significant ($P < 0.05$) (40.63 ± 29.41), when compared to the vehicle control (119.25 ± 61.82). Administration of flumazenil to mice who received scopolamine led to a significant increase ($P < 0.05$) in transfer latency (99.88 ± 60.15) as compared to scopolamine alone group [Figure 1].

In the present study, flumazenil did not improve memory retrieval in normal mice. This can be due to less prominent role of endozepines in memory retrieval process in normal brain. Scopolamine, a known amnestic agent, was used as a disease control. Flumazenil pretreated mice showed a significant improvement in memory retrieval as shown by the increase in transfer latency on test day when compared with scopolamine alone group. Although the specific nature of the facilitation of learning/memory by flumazenil was not directly addressed in our study, the experiments performed did provide some clue as to what phases of memory processing, (i.e., acquisition, storage, or retrieval) could have been facilitated by benzodiazepine receptor antagonism. Previously a study done by Lal *et al*. had shown that flumazenil when administered before acquisition facilitated learning and retention of memory.[11] Because in the present study, flumazenil was not administered during the acquisition phases (training session) and was given just before test session, it can be proposed that it affected retrieval of memory. The results indicate the possible benefit of flumazenil for improving memory retrieval in diseased conditions such as Alzheimer's dementia. Inclusion of a rivastigmine treated group for comparison would have added more information on the magnitude of effect of flumazenil in comparison with rivastigmine. This is limitation of the study.

Since the training was discontinued when the mice had reached a fixed level of time limit (30 s), the inherent differences in acquisition between flumazenil-treated and disease control mice during training phase probably had minimal influence on subsequent recall. Instead, improved recall after administration of flumazenil may reflect facilitation of memory retrieval processes occurred at the time of challenge with scopolamine. Although flumazenil is predominantly a specific antagonist at benzodiazepine receptors, it may act as a weak agonist or inverse agonist after relatively high doses.[9] It is unlikely that the memory-facilitating effects resulted from an agonist (diazepam-like) action of flumazenil because such an action would be expected to impair, not facilitate, memory. Thus, it is inferred that facilitation of memory retrieval by flumazenil is related to antagonism of diazepam-like endocoids rather than agonist actions. The experimental paradigm employed in the present study experiment involved aversive stimulation, and we have not yet tested the effect of flumazenil in other learning or memory contexts (e.g. rewarding situation). Since flumazenil prevented scopolamine-induced amnesia, it can also be postulated that its effect is due to possible modulation of the cholinergic system by GABAergic mechanisms. It would be of interest to study the possible involvement of brain cholinergic system in the memory enhancing the effect of flumazenil. This is because cholinergic system is involved in the pathophysiology and also its modulation is the primary target for drugs used in the treatment of AD.[1] Regardless of mechanisms involved in mediating effects of benzodiazepine antagonists on memory, our study provides preliminary evidence that "benzodiazepine receptor" could be an important new target for development of drugs against AD.

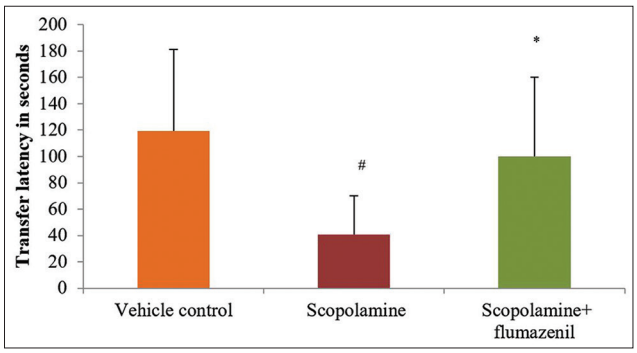

Figure 1: Values represent mean±SD, $P<0.05$: scopolamine *Vs* vehicle control, $P<0.05$: scopolamine+flumazenil *Vs* scopolamine

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Conflicts of interest

There are no conflicts of interest.

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