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Midazolam but not propofol for sedation impairs long term spatial memory in mice

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Abstract

Aim: Sedation may alter memory and learning. This study is designed to investigate the effects of short-acting sedative agents, propofol and midazolam, on learning and memory in mice.

Materials and Methods: Thirty male, adult Swiss-albino mice were randomly assigned into three groups as control, midazolam and propofol groups (n=10, for each). Experiments on learning and memory were started 72 hours after the intraperitoneal injections. Learning and short term memory development in mice were evaluated by Morris Water Maze conducted for five consecutive days, and on the 19th day long term memory formation was evaluated by the same experimental set up. Time for the mice to find the escape platform (latency), total distance covered and swimming speed were recorded and evaluated statistically.

Results: Propofol and midazolam administrations were found to increase the latency. In addition, midazolam was found to impair memory development, when compared to other two groups ($p < 0.01$). Swimming speed and distance covered as indicators of the motor activity decreased gradually from the beginning, however there was no statistically difference between the groups ($p > 0.05$).

Conclusion: Use of midazolam and propofol prolonged the latency on the first three days. Midazolam was also found to affect the memory development negatively.

Introduction

General anesthesia affects brain functions at all levels, including neuronal membranes, receptors, ion channels, neurotransmitters, and cerebral blood flow and metabolism [1]. Culley et al. showed that the impairing effect of general anesthesia on spatial memory lasts for 2 more weeks after anesthesia in aged rats [2]. Although the amnestic effect of general anesthesia has been well established [3], the relationship between cognition and general anesthesia appears to attract researchers as a novel field with growing interest [4]. Consequently, the upper brain functions like cognitive activities show changes of varying degrees during the post-anesthesia period. In anesthesia management, rapid and complete recovery of cerebral functions is the main target of anesthesiologists. Evaluation of the postoperative cognitive functions may help to investigate mental changes or recovery levels of the patients after anesthesia and/or surgery [5-7]. Postoperative cognitive dysfunctions are seen in a wide variety of clinical settings, ranging from concentration impairment to delirium [1, 2, 4].

Sedation with propofol and midazolam has been widely used in clinical settings, such as units of endoscopy, dentistry, radiology, cardiology, etc. Despite this there have been scant data on the effects of low dose propofol or midazolam on learning and memory development. Our aim in this experimental study was to investigate the effects of propofol and midazolam, on learning and memory in mice.

Materials and Methods

This study was approved by Baskent University Medical and Health Sciences Research Committee and The Ethical Committee for Experimental Research on Animals (Project number: DA 09/43) and was supported by Baskent University Research Fund.

Animals

The mice enrolled were obtained from Baskent University Experimental Animal Production and Research Center.

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Fifteen months-old, thirty male Swiss-albino mice, weighing 35.7-53.4 g were used to investigate the effects of propofol and midazolam administration on learning and memory formation. After a week of acclimation period in the laboratory with standard feeding and care, mice were randomly assigned into three groups as control group (n=10), propofol group (n=10), and midazolam group (n=10).

Morris water maze

Morris water maze (MWM), made of black fiberglass (circular, diameter 120 cm, height 60 cm, depth of water 40 cm) filled with water with constant temperature of 26 ± 2 °C was used to evaluate the learning and memory functions. Several cues to help the mice for orientation of different colors, shapes and sizes were placed on the walls surrounding MWM. The walls were named in accordance with the four main directions (East, West, North, South) A circular escape platform with 10 cm diameter, was placed at a certain point (33 cm to the maze wall), and kept 1 cm above and 1 cm below the water surface during the periods of training and assessment of learning respectively. The platform was covered with a black fleece cloth, so that mice could easily claw and feel safe.

Study protocol

All mice were trained for three subsequent days to get acclimated to the MWM protocols. On the fourth day, the agents (propofol 100 mg/kg/1 mL, midazolam 4 mg/kg/1 mL) or placebo (physiological saline, 1 mL) were injected intraperitoneally to the mice that were fasted for the preceding 6 hours to mimic the clinical conditions. The loss of writhing and pedal retraction reflexes was accepted as indicators of adequate level of sedation. To avoid the confounding acute effects of the drugs, and to allow an adequate period for elimination, MWM experiments were started 72 hours after the injections. MWM experiments to assess learning and short-term memory formation was conducted for five consecutive days, between 09:00 a.m.-17:00 p.m. During the study, investigators and the environment were kept stable and constant in terms of temperature, color, humidity, odor and sound. At the end of each trial, mice were dried with towel and kept warm in a heated box. The trial was initiated by lowering the mouse to the zero point on the surface of the water, facing the maze wall. The mice were put into the water maze at four different zero-point locations (named after four main directions; North, South, East and West) with random order each day. Once put into the water tank, the mice were observed via a webcam-attached computer monitor outside the MWM room, and the activity pattern was recorded. The latency for animals to find the escape platform and the total distance they swam were measured by a digital chronometer. Each mouse was allowed to swim for a period of 120 seconds at each trial, if it failed to find the escape platform. If the animal succeeded to find the platform before the cut-off time, the latency was recorded and it was allowed to stay on the platform for 10 seconds to recognize the environmental cues. If the mouse failed to find the escape platform within 120 seconds, it was manually guided to the platform and was let to stay there for 10 seconds to learn the cues. Swimming speed and total distance covered were recorded as indicators of the motor activity of each animal. The animals' movements were recorded by a CCD (Charge coupled device) camera attached to the ceiling. Latency (sec), motor activity (swimming speed, cm/sec) and total distance covered (cm) were recorded and analyzed by a customized MatLab-based tracking program developed by the Biomedical Engineering Department of Baskent University Engineering Faculty. The protocol was lasted in a total period of 32 days, as shown Figure 1.

Statistical analysis

Statistical analyses were performed by using the SPSS 17.0 (SPSS, Statistical Package for the Social Sciences program, Chicago, IL, USA). Compliance with the normal distribution of variables was checked with Shapiro-Wilk test. Friedman's test was used for dependent (within the group, time-dependent) group medians comparison and Kruskal-Wallis analysis of variance was used for independent (between the groups, due to drug use) group medians comparisons. Bonferroni-Dunn's test was applied for multiple comparisons and $p<0.05$ was considered as statistically significant. Data were expressed as arithmetical mean ± standard error of mean in the figures and median values were given in tables in addition for clarity of understanding.

Results

Data regarding the latency, swimming speed and total distance covered in three groups are as follows.

Latency

Latency (time to find the escape platform) values of the groups are shown in Figure 2 and Table 1. Both midazolam and propofol administrations increased latency when compared to the control group, especially in the first 3 days. Increase in the latency in the midazolam group was higher than the other two groups, but this was not statistically significant. Similarly, the data of memory function

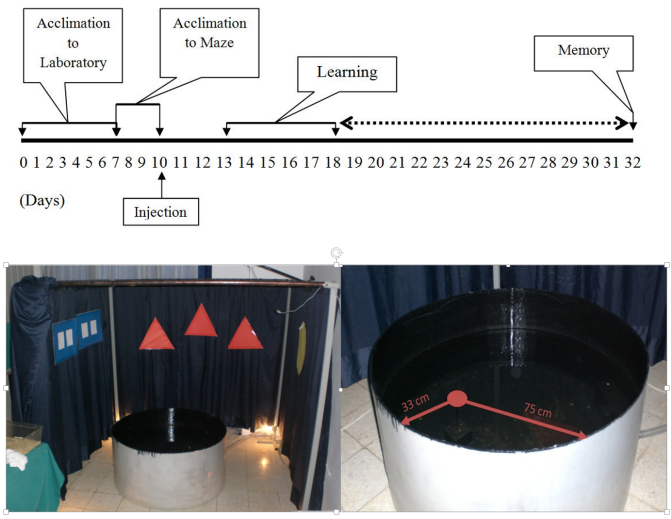

Figure 1. Scheme of the study protocol and picture of Morris Water Maze.

Table 1. Average numeric values of latency (sec), (Median values were given in paranthesis).

Groups	Control	Propofol	Midazolam
Day 1 (sec)	90.50 ± 36.81 (98.00)	60.00 ± 32.27 (60.87)	74.20 ± 30.29 (56.25)
Day 2 (sec)	78.60 ± 31.21 (66.00)	75.00 ± 32.38 (72.38)	91.50 ± 17.31 (94.75)
Day 3 (sec)	79.90 ± 43.03 (8375)	85.40 ± 39.23 (96.50)	114.10 ± 17.16 (120.00)
Day 4 (sec)	88.40 ± 40.16 (101.25)	77.90 ± 41.33 (73.75)	114.50 ± 17.30 (120.00)
Day 5 (sec)	80.60 ± 33.25 (82.00)	84.20 ± 36.86 (120.00)	113.60 ± 13.68 (120.00)
Day 19 (sec)	75.50 ± 38.02 (74.30)	81.00 ± 36.80 (68.50)	117.5 ± 7.90 (120.00)*

* Significantly different when compared to the Control and Propofol groups (p<0.05).

Table 2. Average numeric values of swimming speed (cm/sec), (Median values were given in parenthesis).

Groups	Control*	Propofol**	Midazolam**
Day 1 (cm/sec)	11.60 ± 4.04 (10.18)	14.20 ± 5.02 (12.90)	11.90 ± 3.76 (12.23)
Day 2 (cm/sec)	7.90 ± 2.49 (6.60)	10.60 ± 5.79 (8.75)	7.20 ± 2.95 (6.95)
Day 3 (cm/sec)	5.90 ± 3.04 (7.00)	5.40 ± 4.08 (2.83)	3.20 ± 1.40 (2.57)
Day 4 (cm/sec)	6.60 ± 4.63 (4.27)	7.30 ± 5.33 (6.69)	3.30 ± 1.65 (2.65)
Day 5 (cm/sec)	5.60 ± 3.28 (4.67)	7.20 ± 6.12 (2.93)	2.52 ± 1.18 (2.22)
Day 19 (cm/sec)	4.50 ± 3.33 (6.50)	4.10 ± 3.47 (2.01)	2.30 ± 1.01 (1.70)

*Significant gradual decrease was seen in the Control group (p<0.05) **Significant gradual decreases were seen in the Propofol and Midazolam groups (p<0.01).

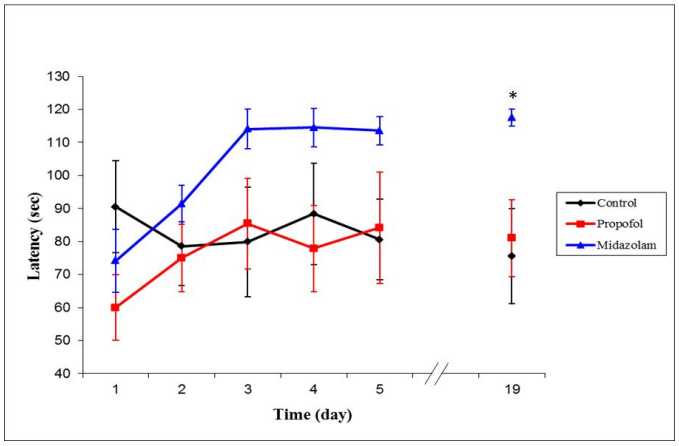

Figure 2. The effects of midazolam and propofol on latency (time to find the escape platform) at Morris water maze * p<0.05 (vs control group).

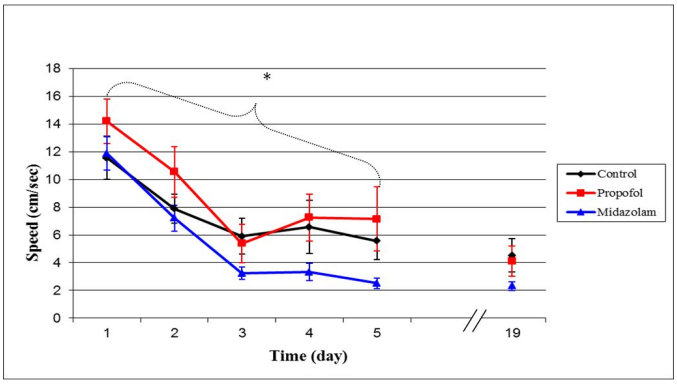

Figure 3. The effects of midazolam and propofol on average swimming speed at Morris water maze. * p<0.01 (for propofol and midazolam groups), p<0.05 (for control group).

assessed on day 19 with the same method, showed that midazolam impairs the memory retention when compared to the other two groups (Latency values, 120 sec (midazolam) vs 74.3 sec (control) and 68.5 sec (propofol), p < 0.05 for all).

Swimming speed

In all three groups significant decrease was observed in swimming speed with time, but there was no significant difference between the groups in this respect (Figure 3, Table 2).

Total distance

There was significant decrease in total distance swam in all groups. But there was no significant difference between groups in this respect (Figure 4, Table 3).

Discussion

There have been several reports on the effects of anesthetics on cognitive functions in the literature [5, 8-12], but the number of the studies about effects of anesthetic agents used for sedation on learning and memory are rare [13]. The aim of the present study was to investigate the effects of propofol and midazolam, drugs used for sedation, on learning and memory functions in order to contribute to the optimization of the sedation process, a widely used medical procedure in daily practice. We showed that propofol and midazolam administration increased the latency at the first three days, indicating that learning and short-term memory formation is partly impaired by these drugs. However, only midazolam prolonged the latency on the retention trial, indicating the negative effect of midazolam on long term memory consolidation compared to

Table 3. Average numeric values of total distance (cm), (Median values were given in parenthesis)

Groups	Control*	Propofol**	Midazolam**
Day 1 (cm)	925.70 ± 402.78 (818.13)	735.20 ± 356.70 (725.95)	766.30 ± 228.31 (669.10)
Day 2 (cm)	525.50 ± 149.70 (522.98)	600.60 ± 233.02 (534.56)	543.60 ± 271.00 (426.48)
Day 3 (cm)	358.80 ± 172.60 (317.87)	329.50 ± 113.46 (301.58)	365.50 ± 127.23 (308.60)
Day 4 (cm)	447.20 ± 238.52 (316.51)	364.10 ± 191.00 (265.14)	357.00 ± 76.87 (317.62)
Day 5 (cm)	330.50 ± 78.07 (280.94)	349.90 ± 142.06 (347.67)	274.00 ± 132.56 (255.99)
Day 19 (cm)	232.40 ± 67.22 (202.00)	203.40 ± 72.90 (202.20)	262.50 ± 121.75 (202.80)

*Significant gradual decrease was observed in the Control group (p<0.05) **Significant gradual decreases were observed in the Propofol and Midazolam groups (p<0.01).

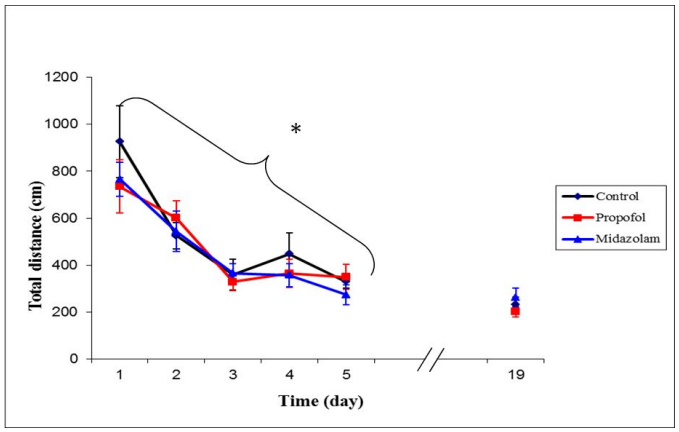

Figure 4. The effects of midazolam and propofol on total distance swum at Morris water maze. * p<0.01 (for propofol and midazolam groups), p<0.05 (for control group).

control group. Indicators of the motor performance of the mice were not affected by the drugs used.

A conventional sedation practice should aim saving cognitive functions of the patients as well as providing comfort, cooperation, stable hemodynamic profile and airway protection. But requirements for the sedatives or analgesics vary depending on many reasons, such as individual pharmacokinetic and pharmacodynamic features, characteristics of the interventions, sensitivity for sedative drugs or targeted depth of anesthesia. The present study was planned to investigate the effect of sedation on cognitive functions, such as learning and memory development. Two most widely used agents, propofol and midazolam, were used to induce sedation. Although using a single level of sedation is a limitation of the present study, it was necessary due to ethical concerns.

Morris water maze was preferred as an experimental set up for evaluating the process of learning and memory due to the absence of a fixed formula for escape and lack of allowing for the formation of hints with repeated usage. Mice are natural swimmers and swimming does not create a stress on mice but, when they find a secure platform while swimming, they prefer staying on it. Accordingly, Morris water maze's rationality is based on this mechanism. In this study, learning and memory functions of mice were evaluated with the time of finding the escape platform (latency) and motor activities of mice were evaluated with total distance swam and swimming speed.

Several clinical and experimental studies have pointed out the age-related variability of anesthesia and sedation affecting cognitive functions [1, 2, 14]. For example, Culley et al. showed that aged rats subjected to general anesthesia without surgery were less capable of learning and performing a spatial memory task than the control rats that were not anesthetized. The authors reported that the impairing effect of general anesthesia on the performance on a spatial memory task in aged rats remains for at least 2 weeks following anesthesia [2].

All the data of the present study showed that, latency values were prolonged on the first three days and remained stable at this level for the 4th and 5th days, in propofol and midazolam groups. The effect of midazolam was found to be more marked. We, therefore suggest that propofol and midazolam administration impairs learning and short-term memory development.

Memory consolidation was also evaluated on the day 19 by a retention MWM trial revealing the impairment in long term memory function in midazolam-administered mice. Latency values were significantly greater in the midazolam group than that of the control and propofol groups.

Data from the previous studies on the effects of propofol on memory are controversial. Similar to our results Lee et al. reported that in aged rats, propofol anesthesia did not modify the memory functions [15]. Conversely, Veselis et al. showed that propofol anesthesia altered the memory functions [16]; moreover Pain et al. revealed that even non-sedative doses of propofol anesthesia (9 mg/kg) impaired the memory without affecting the motor activity in rats [13]. They stated that the effects of propofol on memory are independent of sedative and anxiolytic effects and this effect is similar to the low anxiolytic and non-sedative doses of midazolam.

There are both clinical and experimental data regarding the variability of the effects of anesthetics and sedatives on cognitive functions with age. For example, it has been shown both in humans and in rats that aging causes worsening to a variable degree in cognitive functions including spatial learning and memory [17, 18]. In a multicentric international study performed on 1218 patients over age 60, postoperative cognitive dysfunction was reported in 25.8% of the patients in the first week and 3 months later in the 9.9% of them [8]. A decrease occurs in controlling the motor and reflex responses by age. Motor performance disturbances are caused by the neurodegenerative changes in the striatal dopamine system. Shukitt-Hale et al. per-

formed various tests on rats of 6, 12, 15, 18 and 22 months of age in order to test the performances and spatial learning and to investigate when exactly these changes start in animals. They showed that spatial learning disturbances started in rats of 12 and 15 months old [19]. In the present study we used 15 months old mice. This fact points out that the impairment in learning and memory may not only be due to the drugs given, but also to the age of the mice.

When the learning curves obtained in this study are far investigated, the deterioration of propofol and midazolam curves were expected results. However, in the control group, an increase in the curve on 4th day after a significant decrease in the first three days was unexpected. This situation can be explained by depression development or insensible changes in the environmental conditions. As Holscher et al. stated, in animals to which Morris swimming test was performed, the deterioration of learning performance may not only be related with spatial learning impairment [20]. Any condition which makes the subject uncomfortable, afraid or anxious may affect learning performance. Geretsegger et al. have defined this situation as a depression model and they attributed the inconsistent data to the possible structural and functional damage in the hippocampus [21]. Dong et al. found out that the depressed rats had high levels of glutamate in the hippocampus [22]. They suggested that these high levels of glutamate caused NMDA receptor activation and depression. In our study, we tried to avoid any noticeable change in the environment or any factor that may cause depression except for the stress caused by the experiment itself. That's why the decrease in the swimming time and distances in both 3 groups was thought to be attributed to the depression development.

In this study, while investigating the effects of propofol and midazolam on learning and memory, we also evaluated the motor activity by using the criteria like swimming velocity and distance covered by the mice to exclude the possible confounding effect of sedation on motor activity. Both the swimming velocity and distances in propofol and midazolam groups were same with control group.

Since we could not determine any significant change in the parameters indicating the motor performance among the groups, the change in the latency values due to the drug administrations might be attributed to their effects on cognition.

Our study was well designed and performed in accordance with standard protocol. However, being an experimental study, limited number of pharmacological agents, and lack of support with auxiliary methods such as imaging or laboratory tests can be stated as limitations.

Conclusion

In conclusion, in this experimental study, we observed that propofol and midazolam deteriorated learning process, but this deterioration was not statistically significant. However, midazolam caused a significant deficit in memory consolidation. The motor performance of the mice was unexpectedly decreased in both groups. In brief, using these agents for sedation appears not to affect the spatial learning process significantly, however midazolam may exert detrimental effects on long term memory development. To provide further support for our suggestions based on the findings of the present study, further clinical and experimental investigations are needed.

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Ethics approval

This study was approved by Baskent University Medical and Health Sciences Research Committee and The Ethical Committee for Experimental Research on Animals (Project number: DA 09/43).

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