Locomotor differences in Mongolian gerbils with the effects of midazolam administration in the form of eye drops

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Abstract

Background: Midazolam is a sedative-hypnotic agent with amnestic and anticonvulsant properties that can be administrated to mammals through various routes, such as intravenous, intramuscular, oral, intrathecal, rectal, and buccal. Midazolam administration in the form of eye drops through the conjunctiva is not reported in the literature.

Aim: This study aims to demonstrate the possible central nervous system effects of midazolam administration as eyes drops in Mongolian gerbils.

Materials and Methods: Fourteen gerbils were randomly assigned to one of two equal sized groups. The active arm received 2 ml of 10 mg midazolam as eye drops in both eyes. Control group received a total of 2 ml of physiological saline(0.9% NaCl). We subjected the gerbils to an adapted "Open Field" to determine the possible effects on central nervous system of midazolam. Gerbils were allowed to move freely in the open field. Before and after the drug administration, locomotor activities of each gerbil have been recorded. Frequency of loss of righting reflex was quantified.

Results: Conjunctival midazolam administration resulted with the transient loss of righting reflex ($p=0.017$) and suppressed exploration motion ($p=0.018$) in the open field test compared to control subjects.

Conclusions: In the present study, administration of conjunctival midazolam as an eye drop may affect gerbil's locomotor activities and open field behaviors. We argue that, using a sedative and anticonvulsive drug such as midazolam via conjunctival route may be useful in some clinical situations. Therefore, it could be beneficial to develop a new conjunctival formulation of midazolam. Also, there is a need for trials in humans with pharmacokinetic studies.

Key Words: Righting reflex; Conjunctival midazolam, Eye drop, New administration route

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Introduction

Midazolam is a drug with sedative, amnestic, anxiolytic, hypnotic, and anticonvulsant effects [1]. It is being used successfully in a large number of medical procedures and particularly in many anesthesia administrations [2]. Up to this time, usage routes, such as intravenous (IV), intramuscular (IM), oral, intrathecal, rectal, buccal, and nasal were reported [3-5]. Using as eye drops is not reported in the literature.

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Midazolam in the form of eye drops may have many benefits. During an epileptic seizure outside the hospital, having alternative routes apart from intravenously by the non-medically trained person of an epileptic seizure such as relatives of the patient or paramedics; the administration of anticonvulsive agents through nasal, oral, or rectal routes has been appreciated [6]. An anti-epileptic agent administered into the eye would introduce an alternative method to meet such a need. There are publications stating that the addition of intravenous sedoanalgesia drugs to topical anesthesia causes enhanced undesired results during ocular surgery [7]. In these types of surgeries, the aforementioned complications can be reduced via the addition of midazolam to the local anesthetic eye drops. Moreover, in the ophthalmic operations of agitated patients, the incidence of requests for general anesthesia by the surgical team may be reduced. In infants, children, and patients with communication difficulties, using oral or nasal sedative agents can cause irritation of the airways [8], and induce nausea and vomiting. In such situations,

sedation with eye drops may be advisable. Instead of using oral sedative volumes for sedation, which is already known to carry aspiration and hypoxia risks [9], using the sedatives as eye drops may be a reasonable approach. This can be a helpful way for non-medical staff safely administers a sedative drug. This study is an experimental research about the effects of midazolam administration in the form of eye drops on locomotor activities of Mongolian gerbils.

Methods

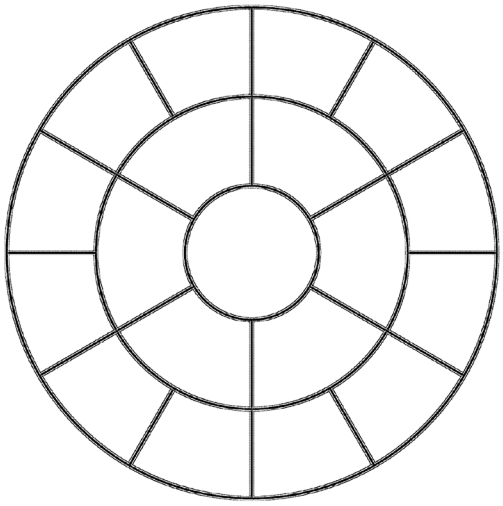
Before the experiment began, the Local Animal Experiments Ethics Committee approved our study (approval number 2012/02). We carried out the experiment with 14 Mongolian female gerbils (Meriones unguiculatus) that were obtained from the Abant Izzet Baysal University Experimental Animals Application and Research Center; these gerbils were from 7 and 12 weeks old and had body weights between 80 and 100 g. We housed the animals at a temperature of 23°C and a humidity between 40 to 60 per cent on a 12-hour dark/12-hour light cycle under ad libitum feeding conditions.

We randomized the gerbils into two groups: the midazolam group and the physiological saline (PS) group as a control, with each group consisting of seven gerbils. The midazolam group received a total of 2 ml of 10 mg midazolam (Dormicum 5 mg.ml-1, 3 ml ampoule, Deva Pharmaceutical Preparations, Istanbul, Turkey) in both eyes as eye drops. The midazolam instillation was given as 20 drops per millilitre. During the instillation, the gerbil was restrained by the researcher using two hands, and the eyelids were opened by an assistant. To allow an opportunity for the absorption of midazolam by the conjunctival sac, the eyelids were released for two seconds after each droplet was administered. We optimized the active conjunctival dose of midazolam in our preliminary unpublished data with Professor Erol Ayaz (Director of the Animal Research Laboratory, Abant Izzet Baysal University) Different doses and concentrations of midazolam were tested to find the most effective level. Two 5- and 10-mg dosages of midazolam were tested for this purpose with concentrations of either 1 mg/ml or 5 mg/ml. Additionally, in the preliminary study, the low pH (3) of the parenteral formulation of midazolam did not cause any local irritation or harm to the gerbil's eyes.

During the application, liquid absorber tissue was used to cover the head and encircle the eyes of the gerbils to prevent the medication from leaking into their nose and mouth. In this way, the gerbils were also prevented from receiving the medicine orally while preening and cleaning themselves afterwards. Using the same steps, 2 ml total of the PS (0.9% NaCl) eye drops were administered to the seven gerbils in the control group. Both eyedropping processes took approximately 60 seconds. Randomization was accomplished by tossing a coin.

We subjected the gerbils to an open field test to measure the possible locomotor effects of midazolam [10]. A closed round cage 33 cm in diameter was divided into 19 equal fragments radially. The base drawing plan for the open field is shown in Figure 1.

Figure 1. The basal scheme for "Open Field Area".



The bottom surface of the open field was made of glass, and the sidewalls were constructed of metal.

All gerbils in both groups were kept in the experiment room for adaptation purposes for 30 minutes before the midazolam and PS administrations. After the adaptation process, randomly selected gerbils were released to the open field, and their locomotor activities were recorded by a camera (Samsung HMX-T10) for six minutes before any substance were dropped into their eyes. After the midazolam and PS eye drops were administered, each gerbil was released into an ordinary cage for five minutes to observe any possible effects of the drug. Then, each gerbil was placed in the open field area, and their locomotor activities were recorded on camera for six minutes. Because the gerbils exhibited extremely rapid movements, recordings were made with a resolution of 720 x 576 pixels and a speed of

50 sq/sec. The open field area was cleaned after each animal to eliminate the scent in preparation for the next gerbil. The camera recordings of the animals were monitored, and the effects of the administration of midazolam and PS on the gerbils' locomotor activities were assessed. All recorded flicks of the animals were watched, and assessments of the locomotor activities of the gerbils were made by a researcher who was blinded to the randomization. The researcher had been entrusted with an electronic counter and a chronometer to transcribe the locomotor activities of the gerbils from the recorded video materials. These activities were identified as seconds in terms of elapsed time and the number of movements.When the gerbils were observed for the control purpose before the midazolam and PS administrations in the cage, they exhibited three main measurable and different movements. The observed locomotor differences after the medication administration in the adapted open field test were identified as measurable and comparable parameters. These were number of standing (NoS) episodes, duration of standing (DoS), and number of losses of the righting reflex (LoRR). NoS can be defined as the count of the exploratory behaviour of standing of the animal on its rear feet with or without leaning against the wall of the open area. DoS was the total duration of the exploration behaviour in six minutes.

Finally, LoRR was an involuntary tumble down on the animal's hip or body. We assumed the transient loss of the righting reflex to be an effect of the medicine because it had also been used to demonstrate the narcotic effect of ethanol in previous studies [11]. One month after the experiment, the gerbils' eyes were scheduled for an examination by a veterinarian and an ophthalmologist to determine any adverse effects of the study.

The LoRR and NoS parameters were not distributed normally, so nonparametric tests (Wilcoxon Signed Ranks Test and Mann Whitney U test) were used for the LoRR and NoS parameters. For the DoS parameter data, in-group comparisons were performed using a paired samples T test, while the inter-group analysis was conducted with an independent samples T test. A p-value less than 0.05 was considered statistically significant.

The sample size of seven was calculated by using the G*Power 3.1.5 (Heinrich Heine University Düsseldorf) power analysis software. We used the LoRR parameter to estimate the number of gerbils in a group as a main goal of the study. In a normal population of gerbils without the influence of medication, spontaneous transient loss of the righting reflex would have never been seen. As a result, it was reasonable to obtain one or more (for example, three) numbers of a mean value of transient loss of righting reflex parameter for estimation. Therefore, we used power of the test as 0.95, the α error as 0.05, the mean of the difference as 3, and the standard deviation of difference as 2 after the program had determined the group size as seven. Statistical calculations were performed using the Statistical Package for the Social Sciences (SPSS) version 11 (SPSS, Chicago, IL, US).

Results

The average values before and after eye drop administration of identified locomotor activities in terms of time and numbers are given in Table 1.

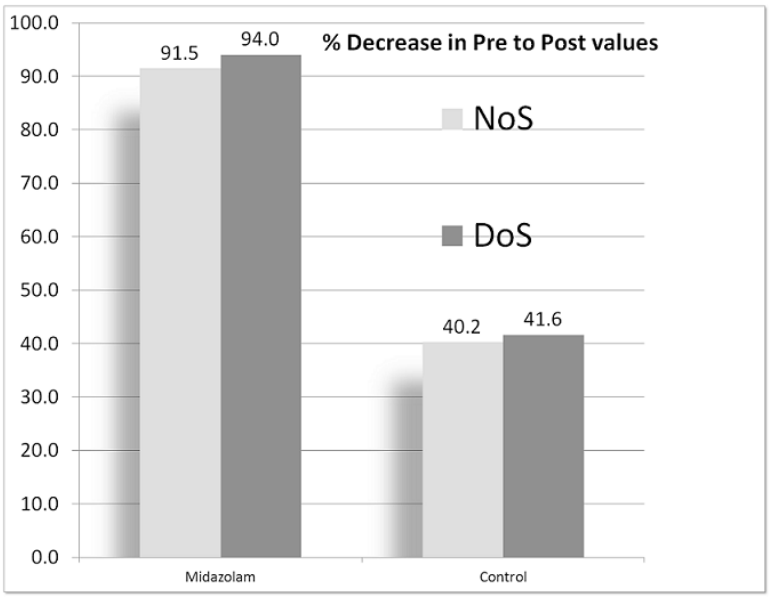
Table 1. Mean values and SD's of measured parameters: before (pre) and after (post) eye drops.

*: P values are given in the text

Results*:	Midazolam		Control	
	Pre	Post	Pre	Post
Number of Standing	127.1 ±31	10.9 ±15	155.1 ±13	92.7 ±34
Duration of Standing (seconds)	193.3 ±73	11.6 ±10	163.1 ±22	95.3 ±40
Number of Loss of Righting Reflex (LoRR)	0	10.4 ±10	0	0

LoRR was observed only in the group that received midazolam (p=0.017). The NoS and DoS values representing exploration movements decreased in both groups. For the midazolam group, the p-values of NoS and DoS were 0.018 and <0.001, respectively (DoS: 95%CI: 119 to 244). The p-values for the PS group were 0.018 and 0.008, respectively (DoS: 95%CI: 23 to 100). However, the decrease in NoS and DoS parameters in the midazolam group was found to be greater than the decrease in the PS group (NoS p=0.017 and DoS p=0.002), as illustrated in Figure 2.

Figure 2. Comparison of % decrease in Pre to Post values of NoS and DoS parameters between groups



An inter-group comparison of NoS and DoS parameters after eye drop applications produced the same p-values of <0.001 for both (DoS: %95CI: -174 to -100).

One month after the experiment, biomicroscopic examination of the gerbils' eyes was found to be within normal limits by a veterinarian and an ophthalmologist. We also could not find any signs of visual dysfunction. There was no difference between the groups in behaviour just after and during the instillation process, and no signs of corneal or conjunctival injury were observed.

Discussion

Among the three parameters, only the transient loss of the righting reflex parameter was possibly attributable to the effect of midazolam. We used the control group to demonstrate that the transient loss of a righting reflex effect of midazolam was not related to corneal contact by a liquid or due to a pH difference. We measured the pH level of the midazolam solution as 3, but this low pH level did not cause any permanent injury, mucoidal discharge, or excessive disturbance to gerbils. During the experiment, especially before the administration, the gerbils' locomotor activities were tracked, and almost all of these behaviours were exploration-oriented. Only after receiving the midazolam did the gerbils experience trouble with the exploration motion of standing on two legs and sniffing. However, in the PS group, the gerbils spent their time standing more frequently on two legs and trying to clean their soaked heads with their front legs, since they could not reach by licking. In the PS group, this decrease in exploration motion was probably related to the cleaning pauses and not due to an effect on the central nervous system by the administered fluid. If a gerbil from the control group had wanted to rear up or sniff, it could have successfully done so. In contrast, gerbils in the midazolam group experienced a loss of balance when they attempted to rear up, and they did not succeed in most instances. Therefore, we considered the transient loss of the righting reflex explicitly observed only in the midazolam group to be powerful evidence of the drug's effectiveness.

Our primary purpose for this study was to determine if midazolam administration through the eyes exhibits clinical effects. All experimental projects requiring possible invasive processes(intra-cardiac blood sampling

etc.) and processes that may endanger the life of the animals are on hold for future experiments in terms of animal ethics.

It could be claimed that the number of standing and duration of standing methods we used to measure animal behaviours(locomotor activities) represent the same behaviours, so recording both values was unnecessary. Actually, characteristics of gerbil behaviours varied; for example, one gerbil made frequent and short waits on two legs while another attempted fewer but longer stands on two legs. Therefore, both values were considered complementary to each other and were recorded.

We determined the active dosage to be 100 mg/kg. As occurs in all topical conjunctival drug delivery administrations, only 20% of the medicine is expected to be absorbed [12].

Additional studies are necessary to be able to generate a more significant dose-response curve, by using different concentrations of midazolam through conjunctival route. It would be needed the formulation which is specifically produced for conjunctival route.

The other important issue is that, such kind of drug administration has possible side effects. Williams described the side effects of the combination of the xylazin-ketamine, as corneal ulceration. They attributed that effect to, staying open for a long time of the eyes [13]. Turner and Albassam, in their studies on rats in 2005, analyzed midazolam, as well, but the most responsible agents for ocular injury were the xylazin-ketamine combination [14]. We kept the gerbils under observation for one month, and then they were assessed by a veterinarian and ophthalmologist. The results of their evaluation indicated no corneal involvement or opacity, and the gerbils were found quite healthy.

Fisher and Chen specified that the oral route is a condemnable application and emphasize the necessity of the new administration methods in anti-epileptic treatment [15]. Anderson and Saneto mentioned the stress of midazolam administration through buccal and intranasal routes and the importance of developing alternative routes [16].

As a support for our secondary hypothesis, Sury and colleagues defined that sedation application on children for radiologic interventions is a serious practice in the sense of skill and decision-making. They stated the number of pediatric anesthetists who are capable of decreasing the risks of sedation will never meet the demand so that, safe methods should be used by non-medical staff [17]. Pitetti and colleagues also supported this approach [18]. Midazolam in the form of eye drops may contribute to solving this problem due to the possible safe administration and feasible titration of dosage.

Katz and colleagues, in their studies on 19,250 cataract surgery patients in United States (US) and Canada, between 1995 and 1997 found that, before additional IV agents were administered; the incidence of undesired cases in topical anesthesia or local anesthesia was 0.13% and 0.78%, respectively. The same rates increased significantly to 1.18% and 1.20% with the application of sedatives and analgesics intravenously [19]. Conjunctival route may be a safer way of administration in these conditions.

This study proved that, the administration of a 10 mg/2 ml dose of midazolam through eye instillations bilaterally for 60 seconds to Mongolian gerbils (varying weights from 80 – 100 g), caused a significant transient loss of righting reflex , an extreme decrease in exploration motion in the adapted Open Field Test. We argue that, using a sedative, anticonvulsive drug such as midazolam via conjunctival route may be beneficial in some certain clinical situations and further studies are necessary to clarify this issue.

The authors declare no conflict of interest in this manuscript.

References

1. Schüttler J, Schwilden H. Handbook of Experimental Pharmacology. Berlin: 2008 Springer-Verlag Berlin Heidelberg 2008.

2. Dundee JW, Halliday NJ, Harper KW, Brogden RN. Midazolam. A review of its pharmacological properties and therapeutic use. Drugs 1984; 28: 519-543.

3. Nordt SP, Clark RF. Midazolam: a review of therapeutic uses and toxicity. J Emerg Med 1997; 15: 357-365.

4. Wolf P. Acute drug administration in epilepsy: a review. CNS Neurosci Ther 2011; 17: 442-448.

5. Ho KM, Ismail H. Use of intrathecal midazolam to improve perioperative analgesia: a meta-analysis. Anaesth Intensive Care 2008; 36: 365-373.

6. Zawadzki L, Stafstrom CE. Status epilepticus treatment and outcome in children: what might the future hold? Semin Pediatr Neurol 2010; 17: 201-205.

7. Sharwood PL, Thomas D, Roberts TV. Adverse medical events associated with cataract surgery performed under topical anaesthesia. Clin Experiment Ophthalmol 2008; 36: 842-846.
8. Chung T, Hoffer FA, Connor L et al. The use of oral pentobarbital sodium (Nembutal) versus oral chloral hydrate in infants undergoing CT and MR imaging--a pilot study. Pediatr Radiol 2000; 30: 332-335.
9. Green SM, Krauss B. Pulmonary aspiration risk during emergency department procedural sedation--an examination of the role of fasting and sedation depth. Acad Emerg Med 2002; 9: 35-42.
10. Choleris E, Thomas AW, Kavaliers M, Prato FS. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci Biobehav Rev 2001; 25: 235-260.
11. Miquel M, Correa M, Aragon CM. Methionine enhances alcohol-induced narcosis in mice. Pharmacology, biochemistry, and behavior. Sep 1999;64(1):89-93.12. Walsh RN, Cummins RA. The Open-Field Test: a critical review. Psychol Bull 1976; 83: 482-504.
12. American Academy of O. Fundamentals and principles of ophthalmology. San Francisco, CA: American Academy of Ophthalmology 2008.
13. Williams DL. Ocular disease in rats: a review. Vet Ophthalmol 2002; 5: 183-191.
14. Turner PV, Albassam MA. Susceptibility of rats to corneal lesions after injectable anesthesia. Comp Med 2005; 55: 175-182.
15. Fisher RS, Chen DK. New routes for delivery of anti-epileptic medications. Acta Neurol Taiwan 2006; 15: 225-231.
16. Anderson GD, Saneto RP. Current oral and non-oral routes of antiepileptic drug delivery. Adv Drug Deliv Rev 2012; 64: 911-918.
17. Sury MR, Harker H, Begent J, Chong WK. The management of infants and children for painless imaging. Clin Radiol 2005; 60: 731-741.
18. Pitetti RD, Singh S, Pierce MC. Safe and efficacious use of procedural sedation and analgesia by nonanesthesiologists in a pediatric emergency department. Arch Pediatr Adolesc Med 2003; 157: 1090-1096.
19. Katz J, Feldman MA, Bass EB et al. Adverse intraoperative medical events and their association with anesthesia management strategies in cataract surgery. Ophthalmology 2001; 108: 1721-1726.