

Effect of zolpidem on functional recovery in a rat model of ischemic stroke

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Abstract

Objective: To evaluate the effects of zolpidem on functional recovery in a rat model of acute ischemic stroke.

Methods: Following ischemic stroke procedures, 42 rats (six in each group) were randomly assigned to receive zolpidem (0.1, 0.25, 0.5, 1.0, 2.0 or 4.0 mg/kg) or normal saline administered intraperitoneally once daily for two weeks. Motor behavioural index (MBI) scores, radial 8-arm maze (RAM) test times and brain MRI scans were obtained 24 hours (Day 1) and two weeks (Day 14) post-procedure. Immunohistochemistry was performed on Day 14.

Results: By comparison with the normal saline group, the 0.5 and 1.0 mg/kg zolpidem groups showed statistically significant improvements in MBI scores and increased numbers of brain-derived neurotrophic factor (BDNF) stained cells over the two week dosing period. By contrast, the 4.0 mg/kg zolpidem group had statistically significantly impaired MBI scores compared with the control group. No differences among groups were found in RAM times or infarction volumes.

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Conclusions: This study in a rat model showed that 0.5–1.0 mg/kg of zolpidem had beneficial effects on behavioural recovery by enhancing neural plasticity without causing any memory impairment in acute ischemic stroke.

Keywords

Zolpidem, insomnia, stroke, recovery, ischemia, rat model of ischemic stroke

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Introduction

Sleep is frequently disturbed after neurologic disorders including stroke.¹ Reports suggest that approximately two thirds of patients with ischemic stroke suffer from symptoms of insomnia in the initial stage² and sleep disturbance is thought to attenuate stroke recovery and neuroplasticity.³ Insomnia after a stroke aggravates brain damage and causes oxidative stress resulting in the formation of reactive oxygen species which may eventually lead to neuronal and cellular damage.⁴ Therefore, an effective sleep management protocol should be an essential part of patient care following a stroke.

Zolpidem, a short-acting and non-benzodiazepine related hypnotic that enhances the gamma aminobutyric acid (GABA)_A receptor function by binding selectively to the omega-1 receptor subtype, is often prescribed for the management of post-stroke sleep disturbance.^{5–7} It is known that hypnotics acting on the GABA_A receptor, especially benzodiazepines, produce anterograde amnesia and can impair daytime cognitive function and synaptic plasticity.^{8–10} In contrast with benzodiazepines, zolpidem has similar hypnotic efficacy but more favourable safety and tolerability profiles.¹¹ Furthermore, zolpidem has been shown, in an *in vitro* study in hippocampal cells, to exert antioxidant and neuroprotective effects against oxidative stress and neuronal death induced by glutamate.¹² During

acute ischemic stroke, cell membrane depolarization, excessive release of excitatory neurotransmitters, including glutamate and Ca²⁺ influx endangers neurons in the ischemic penumbra.^{13,14} One study found that pregabalin, which is an analogue of GABA, reduced excessive release of glutamate and protected neurons against Ca²⁺ overload in ischemic stroke.¹⁵ Not only is zolpidem GABAergic but it also has neuroprotective and anti-oxidant properties.¹⁶ However, one study found that zolpidem inhibited neural plasticity in the acute stroke period.¹⁷

To date, there have been no studies examining the effects, beneficial or harmful, of zolpidem on functional recovery following a stroke. Therefore, the objective of this present study was to evaluate the effects of zolpidem on behavioural function, neural plasticity and memory in a rat model of acute ischemic stroke and to examine possible antioxidant properties.

Materials and methods

Rat model of ischemic stroke

Six-week-old male Sprague-Dawley rats (220–280 g) were housed in laboratory cages in a controlled environment (22.0–24.0°C) and kept under a 12/12 h dark/light cycle with free access to food and water. This study was approved by the Institutional Animal Care and Use Committee of the Medical Research Institute in Kangbuk Samsung Hospital, Republic of Korea

The model of ischemic stroke in the rat was produced by the Longa method,¹⁸ whereby the middle cerebral artery was occluded for two hours and re-perfused. Rats were anaesthetized with 2% isoflurane (1:2 mixture of O₂/N₂O) and heating pads were used to maintain their body temperature 36–38°C. A 3 cm incision was made over the middle of the neck and the left common carotid artery exposed. After separation of the internal and external carotid arteries, a 4/0 nylon filament with a rounded tip was inserted into the bifurcation of the common carotid artery until the nylon tip occluded the common carotid artery. The inserted nylon was removed after two hours of occlusion for the transient focal cerebral infarction.

Behavioural function measurements

To investigate the effects of zolpidem on motor recovery after an acute ischemic injury, rats were randomly assigned to one of seven groups (i.e., 0.1, 0.25, 0.5, 1.0, 2.0, or 4.0 mg/kg zolpidem or normal saline) and treatments were administered by intraperitoneal (IP) injection once daily (i.e., 10 am) for two weeks starting the day after the ischemic stroke procedure (Day 1). Weight was measured on Day 1 and two weeks later (Day 14) and the percentage ratio calculated. Behavioural function was measured by a blinded researcher (K.J.Y.) on Day 1 and Day 14 using the motor behavioural index (MBI).¹⁹ The half-life of zolpidem is 2–3 hours²⁰ and so scores were measured at 3 pm to avoid its sleeping effect. The MBI which has six items (i.e., spontaneous activity, symmetry of the four limbs, forepaw outstretching, climbing, body proprioception and response to vibrissae touch) has a total score range of 3–18 with high scores indicating better behavioural function than lower scores. The ratio in MBI (%) was calculated as the score at Day 14/the score at Day 1 × 100.

Memory function measurements

To assess the effect of the zolpidem on memory function, the radial 8-arm maze (RAM) test was performed at 3 pm on Day 1 and Day 14. The RAM has eight U-shaped horizontal runways (10 × 15 × 50 cm), which were placed radially around a hexagonal platform (10 cm) on the floor surrounded by visual markers. After food restriction for half a day, rats were placed on the hexagonal platform and allowed to find and eat four hidden sugar baits out of eight pellets which were set at the end of each arm. Rats were pre-trained twice daily for five days before the ischemic stroke procedure. All situations including the location of RAM on the floor and the sugar bait pellets were identical. The time taken to find and eat four sugar bait pellets was recorded by one blinded investigator (K.J.Y.). The ratio in RAM (%) was calculated as the time at Day 14/the time at Day 1 × 100.

Brain imaging

MRI scans of the brain were performed on Day 1 and Day 14 to evaluate the change in infarction volume. Rats were anesthetized with an IP injection of 1.0% ketamine and placed on a 4.7 T Bruker Biospec Imager (Bruker medical system, Kalsruhe, Germany). The warm magnetic bore maintained the rats' body temperature. The protocol for MRI imaging included a diffusion weighted image (DWI) sequence (TR/TE = 2000/80 ms) and a T2-weighted image (T2WI) sequence (TR/TE = 4500/80 ms). The slice thickness was 1.5 mm and the matrix size was 256 × 256. The infarction volumes which were measured by a blinded researcher (Y.T.L.) using Paravision 3.0 software (Bruker Biospec, Ettlingen, Germany) were obtained from DWI at Day 1 (V1D) and T2WI at Day 14 (V2W). The ratio in infarction volume (%) was calculated as V2W/V1D × 100.

Immunohistochemistry

On Day 14, rats were anesthetized with isoflurane and perfused transcardially with phosphate-buffered solution. Brains were removed, fixed in 10% formalin for one week, dehydrated in a graded ethanol series, embedded in paraffin, and processed for a paraffin block which was cut in 6µm thick sections. For immunostaining, the sections were de-paraffinized in xylene, rehydrated in a graded series of ethanol, washed with normal saline and microwaved in citrate buffer for 10 min. Hydrogen peroxide was used to block endogenous peroxidase activity which enhanced nonspecific immunoreactivity. Two antibodies, brain-derived neurotrophic factor (BDNF, ab6201, 1/50, Abcam, Cambridge, MA, USA) and inducible nitric oxide synthase (iNOS, ab3523, 1/20, Abcam, Cambridge, MA, USA) were used to evaluate the upregulation of neural plasticity and antioxidant effects, respectively.^{21,22} Immunostained sections were digitized using a 40× objective (Leica DFC 290, Leica, Heerbrugg, Germany) in conjunction with the Leica Application suite (version 3.3.0, Leica). Five views from each area of the peri-lesional site were assessed by a pathologist blinded to the experimental conditions (S.W.C.) and stained cells were counted and averaged.

Statistical analyses

Statistical analyses were performed using SPSS software (PASW Statistics for Windows®, version 18.0. Chicago, USA.). Data were analysed using the Kruskal–Wallis test and a *P*-value < 0.05 was considered to indicate statistical significance. The Mann-Whitney test with Bonferroni correction was used to analyse between group differences if the *P*-value was significant.

Results

In total, 42 rats, six in each group, were randomly assigned to one of seven groups (i.e., 0.1, 0.25, 0.5, 1.0, 2.0, or 4.0 mg/kg zolpidem or normal saline). There were no statistically significant differences among the groups in rat weight or MBI at Day 1. The weights (g, mean ± SD) of the 0.1, 0.25, 0.5, 1.0, 2.0, 4.0 mg/kg zolpidem and normal saline groups were 165.3 ± 10.8, 180.0 ± 17.3, 165.7 ± 12.8, 178.8 ± 41.3, 173.1 ± 12.4, 198.3 ± 38.0 and 191.3 ± 42.3, respectively. MBI scores (mean ± SD) for the 0.1, 0.25, 0.5, 1.0, 2.0, 4.0 mg/kg zolpidem and normal saline groups were 8.0 ± 0.9, 8.0 ± 2.1, 8.0 ± 1.7, 8.0 ± 2.0, 9.2 ± 1.9, 9.2 ± 1.0 and 8.3 ± 1.4, respectively.

For weight ratio, there were no differences among the groups from Day 1 to Day 14. For MBI ratio (%), compared with the normal saline group, the zolpidem 0.5 mg/kg and 1.0 mg/kg groups had statistically significantly (*P* < 0.005) better improvements in scores over the two weeks (Figure 1). By contrast, the group receiving zolpidem 4.0 mg/kg had a statistically significant (*P* < 0.005) deterioration in scores compared with the group receiving normal saline (Figure 1).

For RAM, there were no differences among the groups at Day 1. With the exception of the zolpidem 2.0 mg/kg and 4.0 mg/kg groups which were excluded from the analysis because of incomplete data, there was also no difference among groups in the Day14/Day 1 RAM ratio. Initial RAM times (sec, mean ± SD) for the 0.1, 0.25, 0.5, 1.0 mg/kg zolpidem and normal saline groups were 46.0 ± 2.7, 45.4 ± 1.6, 46.1 ± 2.6, 45.1 ± 2.3 and 48.4 ± 3.1, respectively. RAM ratios (% , mean ± SD) for the 0.1, 0.25, 0.5, 1.0 mg/kg zolpidem and normal saline groups were 61.8 ± 4.3, 62.7 ± 6.1, 61.6 ± 6.2, 63.0 ± 6.0 and 64.7 ± 11.2, respectively.

Brain MRI scans showed that there were no statistically significant differences in

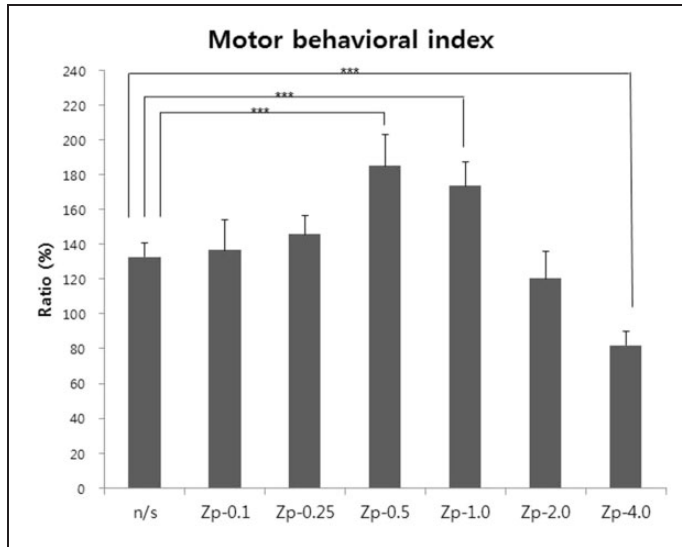


Figure 1. Ratios in motor behavioural index (MBI).

Compared with the normal saline (n/s) group, the zolpidem (Zp) 0.5 mg/kg and 1.0 mg/kg groups had statistically significantly *** ($P < 0.005$) better improvements in scores over the 14 day period. By contrast, the group receiving zolpidem (Zp) 4.0 mg/kg had a statistically significant *** ($P < 0.005$) deterioration in scores compared with the group receiving normal saline (n/s).

infarction volumes among the groups at Day 1 or in the ratio of infarction volumes Day 1 to Day 14 (Figure 2).

Immunohistochemistry showed that by comparison with the normal saline group, the zolpidem 0.5 mg/kg and 1.0 mg/kg groups had statistically significantly ($P < 0.001$) increased numbers of BDNF stained cells (Figures 3a and 4a). However, there were no differences among groups in the expression of iNOS (Figures 3b and 4b).

Discussion

This present study showed that two weeks after a stroke, rats in the 0.5 and 1.0 mg/kg zolpidem groups had significant improvement in behavioural function compared with rats in the control group. In addition, the same two zolpidem dose groups had significantly increased numbers of BDNF stained cells compared with the control

group which indicated upregulation of neural plasticity.²¹ However, there was no difference in iNOS expression among the groups suggesting that functional recovery with zolpidem was not associated with anti-oxidant effects. Although data are lacking to substantiate the hypothesis that zolpidem may have exerted a neuroplastic effect by inhibiting hyper-excitable states caused by neurotransmitters such as glutamate, patients with brain injuries²³ and with Parkinson's disease²⁴ have been shown to benefit from zolpidem in terms of motor recovery.

Interestingly, the group receiving the highest dose of zolpidem (4.0 mg/kg) showed a significant deterioration of behavioural function 14 days weeks after the stroke. While lower doses of zolpidem have a neuroplastic effect, higher doses may be associated with inhibition of the recovery mechanism. For example, a previous study

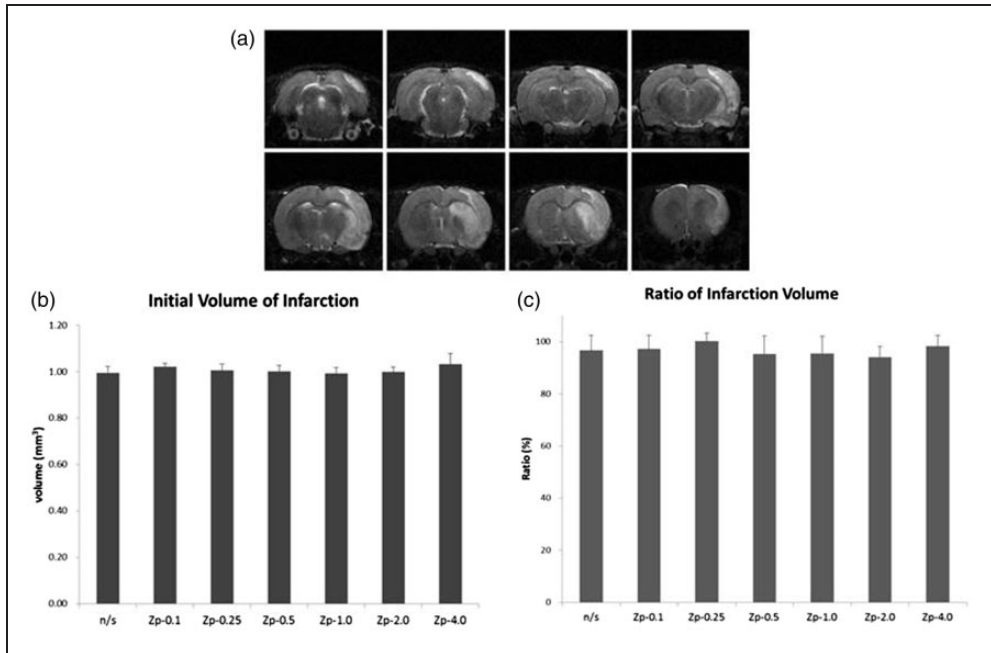


Figure 2. Infarction volumes of the ischemic rat brains.

(a) Lesions in a rat brain (normal saline group) one day after ischemic stroke procedure as shown by diffusion-weighted magnetic resonance imaging.

(b) Infarction volumes at Day I were not significantly different among the treatment groups.

(c) Ratio of infarction volumes Day 14/Day I showed that there were no significant differences among groups.

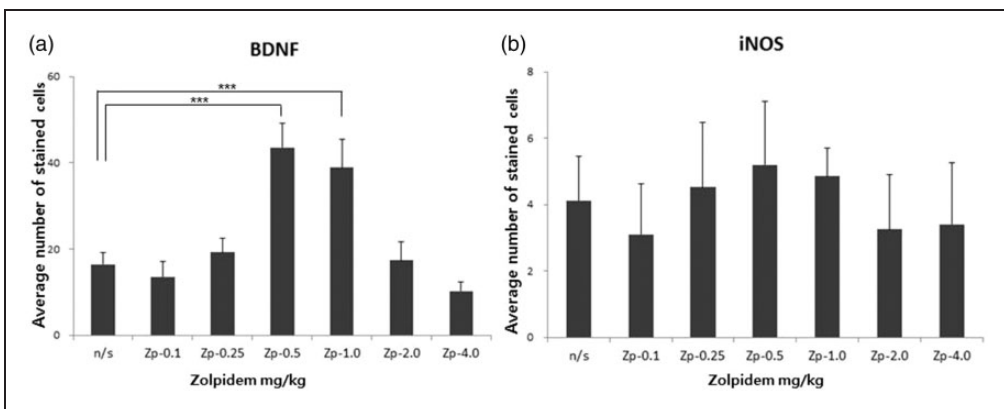


Figure 3. Immunohistochemistry at Day 14.

(a) Immunostained sections using brain-derived neurotrophic factor (BDNF) showed that by comparison with the normal saline (n/s) group, the zolpidem 0.5 mg/kg and 1.0 mg/kg groups had statistically significantly (***) greater numbers of stained cells

(b) Immunostained sections using inducible nitric oxide synthase (iNOS) showed no difference in the number of stained cells among the groups.

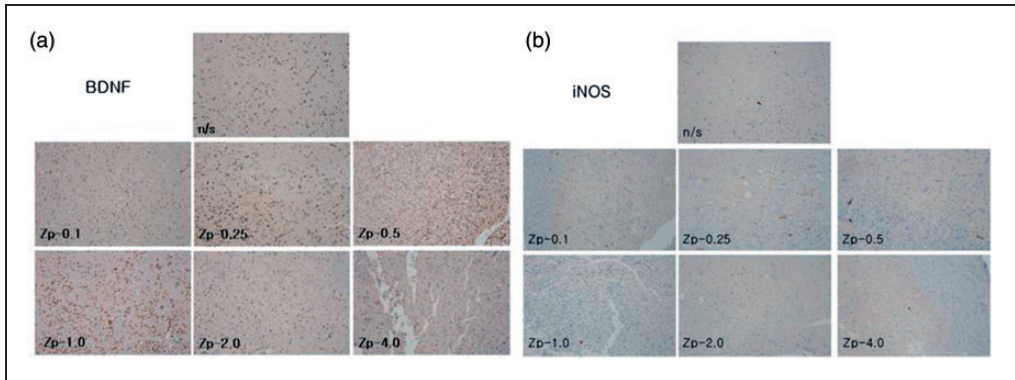


Figure 4. Immunostaining using brain-derived neurotrophic factor (BDNF) and inducible nitric oxide synthase (iNOS).

(a) Examples of immunohistochemical expressions of BDNF at Day 14 from the seven groups (i.e., 0.1, 0.25, 0.5, 1.0, 2.0, or 4.0 mg/kg zolpidem [Zp] or normal saline [n/s]).

(b) Examples of immunohistochemical expressions of iNOS at Day 14 from the seven groups (i.e., 0.1, 0.25, 0.5, 1.0, 2.0, or 4.0 mg/kg zolpidem [Zp] or normal saline [n/s]).

showed that a high dose of zolpidem (10 mg/kg) impaired the cortical plasticity.¹⁷ However, another animal study found that zolpidem 1.0 mg/kg improved functional recovery after stroke.²⁵

No differences among groups were observed in the Day1/Day 14 ratio of RAM, suggesting that the beneficial effects of 0.5 and 1.0 mg/kg zolpidem on behavioural function were not associated with any memory impairment. Previous studies in humans assessing the effects of zolpidem on memory function have produced conflicting results.^{26–28} By contrast with the current study, these previous studies included healthy volunteers and evaluated the immediate effects (i.e., within 24 h) of zolpidem.

No differences among groups were also observed in the Day1/Day 14 ratio of infarction volumes. The lack of effect of zolpidem on infarction volumes may be related to the length of the dosing period. Perhaps a dosing schedule of longer than two weeks is required to achieve an effect of zolpidem on the size of the ischemic lesion.

The study had some limitations. For example, animals were only monitored for two weeks after the stroke. Further studies, with an increased follow-up period are required to confirm the long-term effects of zolpidem. In addition, only rat models with middle cerebral artery infarction were used for the study. Therefore, further studies using other types of brain injuries, such as haemorrhage, are required to investigate the possible neuroprotective action of zolpidem more fully.

In summary, this study in a rat model showed that 0.5–1.0 mg/kg of zolpidem had beneficial effects on behavioural function by enhancing neural plasticity without causing any memory impairment in acute ischemic stroke.

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Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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