Brief Report

Evaluation of Histopathologic Findings and Safety of Intravitreal Ketamine Administration on Vitreoretinal Tissue in Rat Model: A Pilot Study

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Abstract

Purpose: To evaluate the safety and histological findings of intravitreal injection of ketamine in rats.

Methods: Each rat received a total volume of 0.1 ml of ketamine 0.01 mol/L (5 rats as ketamine group) or a total of 0.1 ml of normal saline 0.9% (5 rats as control group) under general anesthesia in a sterile condition. A histology assessment was performed 1 month after the intravitreal injection.

Results: Lens opacity, necrosis, and atrophy of retinal layers and optic disc were not seen in five specimens in the ketamine group and five in the normal saline group. There was no inflammation in the vitreous, retinal layers, choroid, optic disc, and optic nerve in both groups.

Conclusion: Intravitreal injection of ketamine in a special dose has no obvious adverse effect on diverse intraocular tissue.

Keywords: Intravitreal, Ketamine, Rat, Safety

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Introduction

Ketamine is known as an NMDA antagonist agent that has analgesic, anti-inflammatory, and antidepressant effects.[1,2] Ketamine is used only in admitted patients, not in outpatients. The exact mechanisms of this drug and its effects on ocular tissues remain unknown despite its theoretically potential effect as a neuroprotective agent. On the other hand, the neuroprotective effect also has been identified as one of the outcomes of the administration of ketamine that may be related to a reduction in excitotoxicity triggered following an increase in glutamine by ketamine.[3,4] There are a few studies about the effect of ketamine on ophthalmic diseases. In a study, intravitreal administration of ketamine was evaluated in different doses and finally, it was known that the safe doses in rat models were doses up to 0.118 mmol/L.[5] The evaluation of the toxic effect of ketamine was assessed in a short term; only after 7 days by using an electrophysiological test and histology assessment.[5]

Recently, there are many findings about the role of glutamate in the pathogenesis of ischemia.[6] Glutamate plays a role in neurotoxicity resulting in ischemia by activation of NMDA receptors.[7] Ganglion cells, bipolar cells, and photoreceptors in the retina were known as sources of glutamate.[8] Retinal ischemia leads to cell degeneration and activation of the inflammatory cascade.[9] These events are accompanied by

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disturbance of mitochondrial function reduced energy reach to ischemic tissue and lead to optic nerve damage.[10] Utilization of neuroprotective strategy and administration of agents with possible neuroprotective activity may be theoretically beneficial effects in damage reduction caused by retinal ischemia.[11] There are evidences that ischemic damages to tissues can be reduced by administration of NMDA antagonist agents.[12]

Our hypothesis was that intravitreal ketamine could have protective effects on the ischemic retina. However, before administration of ketamine in humans, it was necessary to ensure the safety of intravitreal injection of this agent. Therefore, we decided to evaluate the effect of intravitreal ketamine on retinal tissue for a longer time after 1 month. Thus, the aim of this study is to evaluate the potential toxicity of intravitreal ketamine on retinal cells.

Methods

Ten rats weighting approximately 150 gr (30–60 days old) were housed in a suspended stain-less cage with 12:12 h light: dark cycle with relative humidity (50%–70%) and at constant temperature (22°C–24°C) with free access to water and food were included in our experimental study. The research ethics committee of laboratory animals, Tehran University of Medical Sciences, approved the study protocols (IR.TUMS.1401.007). All parts of the methods were reported in accordance with Animal Research: Reporting of *in Vivo* Experiments guidelines. All rats underwent general anesthesia induced by intraperitoneal administration of ketamine (35 mg/kg) and xylazine (5 mg/kg), and half of the initial dose was used to maintain anesthesia as needed. After maintaining anesthesia, a lid speculum was placed and a drop of topical povidone-iodine 5% was instilled in the right eye of each subject. Then, we entered the vitreous cavity through the superotemporal sclera (2 mm posterior to the limbus) by using a 30G needle (insulin syringe) perpendicular to the center of the globe for avoiding traumatized crystalline lens and a total volume of 0.01 ml of ketamine 0.1 mol/L was injected into the ketamine group in a sterile condition. In the control group, a total of 0.01 ml of normal saline 0.9% was administered intravitreal in the right eye in the same way as ketamine group. Then, a gentamicin drop of 3% was applied after injection in both groups for 5 days every 6 h. All subjects were monitored during the procedure, recovery, and time to ambulation after recovery every week without general anesthesia by using a portable slit-lamp for examination of an anterior segment for hypopyon, corneal edema and opacification, and iris neovascularization (NVI) and significant iris irregularity. To assess the intravitreal toxicity of ketamine, histology of the retina was carried out 1 month later after intravitreal administration. One month after the procedure, all rats were first anesthetized and then euthanized with 100 mg/kg intravenous pentobarbital sodium.

Eyes were fixed with a 10% formaldehyde solution. The eyes were processed and embedded in paraffin after 7 days. Five micrometer vertically oriented tissue sections were cut through the optic disc so that the anterior and posterior portions of each section contained the cornea and optic disc, respectively. The tissue sections were stained with hematoxylin and eosin and examined by light microscopy. The inflammation in intraocular tissues, atrophy, necrosis, and optic disc cupping were evaluated in each sample.

Results

Ten eyes of both groups were evaluated after 1 month for lens opacity, inflammation in different parts of the eyes including the iris, vitreous, retina, choroid, and the optic disc, and finally necrosis in all parts of the eyes by histopathologic evaluation. None of the eyes of the patients showed hypopyon, corneal edema and opacification, lens opacity, NVI, and iris irregularity during follow-up or final examination. The intravitreal injection could result in increasing intraocular pressure. Whereas the effect of increased intraocular pressure on the optic nerve head needs at least 4 weeks for appearing its damage like atrophy on examination, we evaluated the histologic analyses after 1 month that is more accurate and can document the alternation earlier than examination. However, a temporary rise in intraocular pressure might occur. Optic disc atrophy cupping and obvious atrophy in retinal layers were not observed in both groups. No inflammations and no necrosis also were not seen in all evaluated parts of the eye. The pathologic figures of optic discs and retinal layers in both groups are shown in Figures 1 and 2, respectively. As observed in Figure 1, there were no inflammations, necrosis, atrophy, or optic disc cupping in the optic nerve and optic disc of any samples of ketamine or control groups.

As shown in Figure 2, there were no inflammations or obvious atrophy in different retinal layers of samples of five specimens in the ketamine group and five in the normal saline group in histopathological evaluation.

Discussion

Ketamine as an NMDA antagonist agent with potentially neuroprotective effects may be useful in several conditions of eye damage like retinal ischemia. Whereas ketamine is known as hospital medicine, it has not been assessed very well for ophthalmic considerations. There was a study about the utilization of ketamine in ophthalmologic conditions in rat models.[5] There being so, we have decided first to evaluate the safety of the application of ketamine on different parts of ocular tissues like vitreoretinal tissue by using it in the intravitreal administration route.

Our results showed no significant adverse effects including atrophy, inflammations, and optic disc cupping in both ketamine and control groups. Dourado *et al*.[5] evaluated the different doses of intravitreal ketamine and found the safe doses were up to 0.118 mmol/L. Hence, we used the dose of 0.1 mmol/L. They evaluated toxicity after 7 days by using

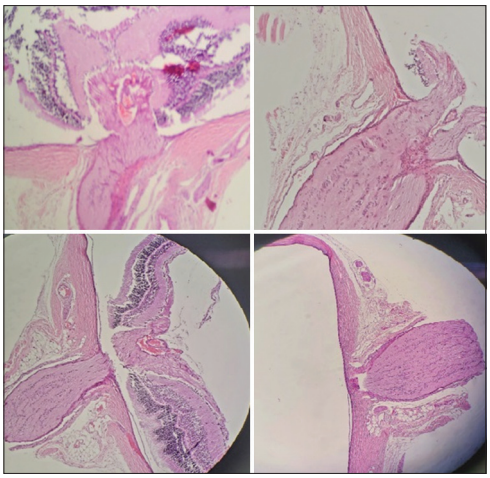

Figure 1: Slide show optic nerve and optic disc of two specimens from ketamine groups (top row) and two specimens from normal saline groups (bottom row). Necrosis, inflammation, gliosis, loss of optic nerve substance, or optic disc cupping were not observed in the case group in comparison with the control group eyes (H and E, ×100)

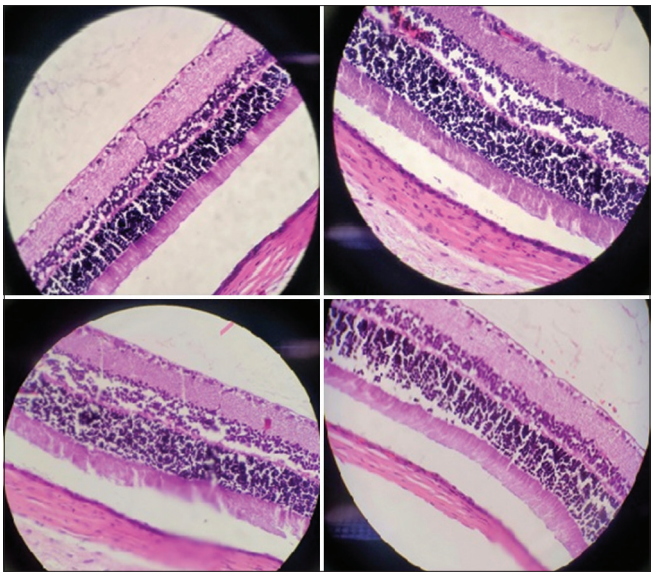

Figure 2: There were no inflammations, obvious atrophy with changes in each layer thickness, number of ganglion cells gliosis, and vacuolization in different retinal layers of samples of ketamine (top row) and saline groups (bottom row) in histological evaluation (H and E, ×400)

electrophysiologic tests and histologic assessments. They found no changes in the dose of 0.118 mmol/L and some reduction in b-wave in dose of 0.237 mmol/L as a marker of retinal damage.[5] Although, histological analyses of both doses showed no changes. We also found no changes in the ketamine and control group after 1 month. As previously mentioned, we preferred to assess after 1 month for detection of optic disc and optic nerve damage like atrophy that needs at least 4 weeks. However, the temporary and reversible effect on retinal layers might be not detected after 1 month. Histopathologic assessment is more accurate than examination and can document the alternation after 4 weeks to some degree. Subsequently, it may be concluded that the application of intravitreal ketamine is safe at special doses based on our results and prior studies.[5]

Following ischemia, a number of harmful events occur, including the formation of free oxygen radicals, malfunction of the antioxidant system, increase in inflammatory cytokines secretion, and extracellular accumulation of glutamate that results in damage to neuronal elements.[13] Following ischemia, disturbance in the balance of excitatory and inhibitory neurotransmitter receptors was reported.[14] Neuroprotection remains an elusive goal for a clinician and preferred administration of neuroprotective agents after the onset of retinal ischemia. In recent years, some studies have been conducted on the protective effects of various agents as neuroprotective against retinal ischemia. One study suggested the neuroprotective effects of intravitreal morphine on ganglion cells of retinal layers during reperfusion study.[15] Furthermore, this study showed that intravitreal morphine had no adverse effects on the retina in histologic studies. A few previous studies concluded that ketamine induces neuroprotective effects on ischemic tissue.[16-18] One study evaluated the toxicity of ketamine in neural stem cells and concluded that it did not have a significant effect when administered with lower than 0.5 mmol/L. Moreover, another study evaluated intravitreal ketamine and suggested that ketamine did not cause any significant damage in doses up to 0.118 mmol/L and they recommended this dose for administration in future studies.[5] They found that ketamine could be able to decrease the wave flatness of electroretinogram parameters after retinal ischemia. Moreover, they showed that intravitreal ketamine had a good effect on the retinal ganglion layer, inner nuclear layer, and outer nuclear layer following retinal ischemia and found ketamine could protect neural cells against injuries and apoptosis. Totally, their study resulted in the fact that ketamine could be administered as an effective neuroprotective drug following retinal ischemia. Given the increasing understanding of the neuroprotective mechanism of ketamine in retinal neuronal ischemia, we hope that this agent can play an effective therapeutic role for retinal ischemia soon.

The limited number of rats is one of the limitations of our study. We assessed the effect of ketamine only after one dose injection and after 1 month. There being so, the potential temporary effects and long-term effects cannot be evaluated in this study. The adverse effects of repeated injections or the effect of injections at different times like 1 day, 1 week, 1 month, and longer time are required in future studies. We only used histology evaluation due to limitation of our facilities. The applications of other modalities like handheld optical coherence tomography may give more exact information about the short-, mid-, and long-time effects of ketamine on different retinal layers.

In conclusion, this study showed an intravitreal injection of ketamine had no significant adverse effects such as inflammation, necrosis, and atrophy on vitreoretinal tissue,

optic nerve, and optic disc. It can be concluded that ketamine may be accounted as a safe agent in special doses. The efficacy of ketamine is required to be evaluated in further studies.

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Conflicts of interest

There are no conflicts of interest.

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