Exploratory Research and Hypothesis in Medicine **2024** vol. 9(4) | 286–293
DOI: 10.14218/ERHM.2024.00029

Original Article

The Aquaporin-4 Inhibitor, N-(1,3,4-thiadiazol-2-yl)-3-pyridinecarboxamide, Inhibits the Glymphatic System: A Laboratory Study

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Received: August 21, 2024 | **Revised:** October 11, 2024 | **Accepted:** October 29, 2024 | **Published online:** November 15, 2024

Abstract

Background and objectives: Aquaporin-4 (AQP4) plays a crucial role in the glymphatic system and is vital for maintaining homeostasis in the central nervous system. This study aimed to investigate the effects of N-(1,3,4-thiadiazol-2-yl)-3-pyridinecarboxamide (TGN-020), a selective AQP4 inhibitor, on glymphatic function and to assess its impact on short-term behavior in mice.

Methods: In this laboratory study, mice were randomly assigned to TGN-020-treated and control groups. We evaluated glymphatic function by measuring the distribution of Evans blue dye in the brain following injection into the cisterna magna. Behavioral assessment of cognitive function was performed using open field and Morris water maze tests. AQP4 protein expression levels were analyzed via immunohistochemistry. Statistical comparisons were conducted using the one-way analysis of variance to evaluate the results among groups.

Results: Our findings revealed that the areas of Evans blue dye in the dorsal ($p < 0.001$) and ventral ($p < 0.001$) surfaces of the brain were significantly reduced in the TGN-020 group compared to the control group, indicating impaired glymphatic function. However, behavioral tests demonstrated no significant short-term changes; the mean distance traveled in the open field was 4,345 cm in the control group and 4,049 cm in the TGN-020 group ($p = 0.5625$), while the mean speed was 2.649 cm/s for controls and 2.868 cm/s for the TGN-020 group ($p = 0.6762$). In the Morris water maze, latency was comparable (36.33 s for TGN-020 vs. 34.89 s for controls, $p = 0.758$). Additionally, no significant differences in AQP4 expression intensity were observed between the two groups.

Conclusions: Our study demonstrates that acute inhibition of AQP4 through a single dose of TGN-020 significantly impairs glymphatic function without inducing short-term behavioral abnormalities in mice. These findings contribute to understanding AQP4's role in the glymphatic system and its potential implications for neurological function.

Introduction

The glymphatic system, a recently discovered para-vascular compartment between the vascular network and astrocyte end-feet, serves as the brain's waste-clearance system.[1] The glymphatic system is a para-vascular pathway between the vasculature and astrocyte end-feet that transports cerebrospinal fluid (CSF) through the brain. The flow of CSF via the glymphatic pathway is essential for eliminating metabolic waste products (such as amyloid-β and lactic acid) from deep within the brain and for delivering nutrients (such as glucose) and drugs into the brain parenchyma. Dysfunction of the glymphatic system may contribute to the pathogenesis of neurological disorders.[2–4]

Aquaporin-4 (AQP4) is the most abundant water channel protein expressed in astrocytes within the glymphatic system, facilitating

Keywords: Aquaporin-4 inhibitor; TGN-020; AQP4; Glymphatic system; Anaesthetic-induced behaviour change; Sevoflurane.

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How to cite this article: Lian C, Zhang T, Cheng P, Li L, Zhao G, Ren X. The Aquaporin-4 Inhibitor, N-(1,3,4-thiadiazol-2-yl)-3-pyridinecarboxamide, Inhibits the Glymphatic System: A Laboratory Study. *Explor Res Hypothesis Med* 2024;9(4):286–293. doi: 10.14218/ERHM.2024.00029.

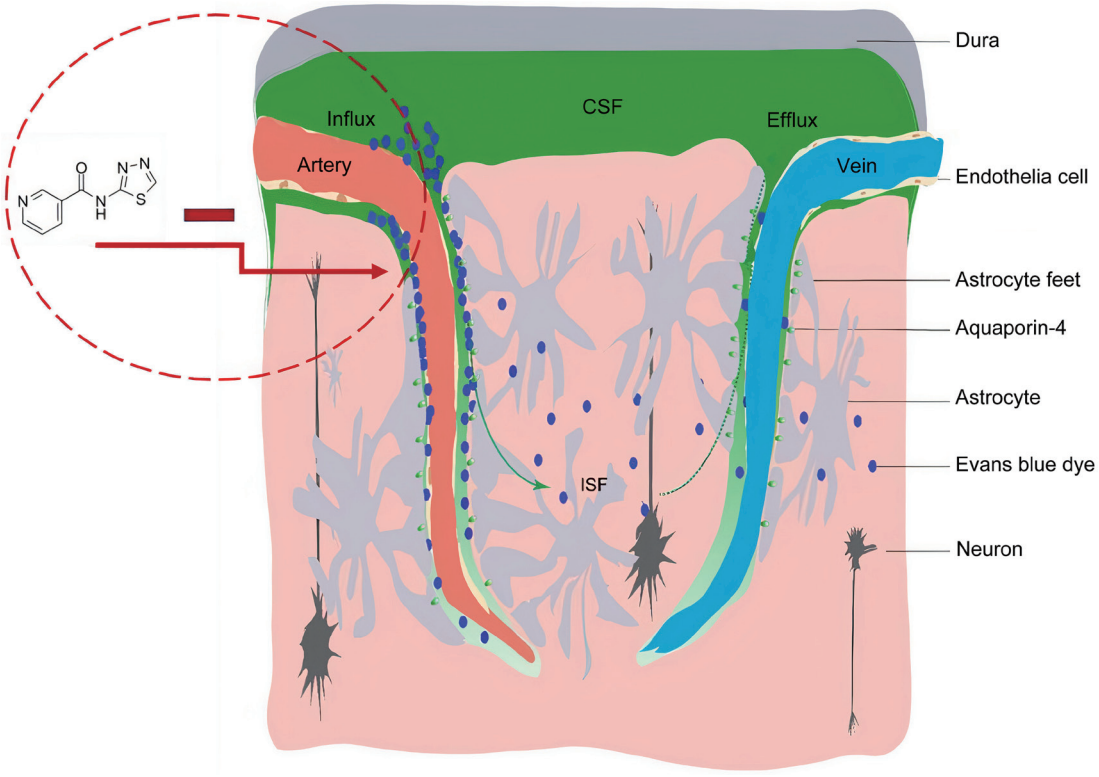

Fig. 1. Schematic diagram of the mechanism of TGN-020's effect on the glymphatic system. CSF, cerebrospinal fluid; ISF, interstitial fluid; TGN-020, N-(1,3,4-thiadiazol-2-yl)-3-pyridinecarboxamide.

rapid convective CSF flow between the perivascular and interstitial spaces in the brain.[2] AQP4 exhibits a polarized distribution on the astrocyte end-feet adjacent to blood vessels under physiological conditions, and this distribution is essential for edema formation and resolution, as well as for the clearance of interstitial solutes in the brain.[5,6] Dysfunction of AQP4 impairs the glymphatic system, leading to toxic protein deposition and cognitive deficits. Gene knockouts, small interfering RNAs, heavy metal ions, and small molecule inhibitors are common techniques for blocking receptors such as AQP4. Due to their safety, small molecule inhibitors may be used in clinical settings. Among these is N-(1,3,4-thiadiazol-2-yl)-3-pyridinecarboxamide dihydrochloride (TGN-020), which has been shown to block AQP4 both *in vitro* and *in vivo* through the intracellular ubiquitin-proteasome system.[7] Some studies have revealed that acute inhibition of AQP4 diminishes brain edema in models of cerebral ischemia and speculate that the mechanism may act as an effective modulator of glymphatic flow.[8] TGN-020 offers a unique and immediate pharmacological approach for inhibiting AQP4, which can be advantageous in acute scenarios requiring rapid intervention. As a small molecule agent, the dosage of TGN-020 can be easily adjusted to meet the needs of different patients. Unlike the delivery limitations of siRNA technology and the permanence of knockout technology, TGN-020 offers greater flexibility and enables personalized treatment regimens. Although the effects of TGN-020 on the glymphatic system have been investigated, current literature primarily focuses on its general inhibitory effects on water channel protein function. Some studies have documented the inhibitory effect of TGN-020 on water channels in the brain,[9,10] but analysis of its specific effects on behavioral or neurological changes is lacking. Therefore, an in-depth study of the effects of TGN-020 on the glymphatic system and neurological function, particularly regarding behavioral changes, will provide important theoretical support for its clinical application.

In this study, we briefly inhibited AQP4 using TGN-020 in normal mice and evaluated the function of the glymphatic system, neurological changes, and AQP4 expression patterns (Fig. 1). We aimed to investigate the effect of AQP4 inhibition on the glymphatic system and subacute outcomes.

Materials and methods

Mice and drugs

All experiments on mice were performed following the guidelines of Jilin University. Male C57BL/six mice (Laboratory Animal Center of Jilin University), aged 12 weeks and weighing 25–26 g during the experiments, were housed under a 12-h light/12-h dark cycle with free access to water and chow. Mice were randomly divided into two groups (n = 6) using a Random Number Table: the control group and the TGN-020 (100 mg/kg) group. This single dose of TGN-020 is based on previous literature.[11,12] TGN-020 (D&C Chemicals, China) was dissolved in dimethyl sulfoxide (DMSO) to increase its solubility and then diluted with sterile phosphate buffered saline to achieve a final concentration of 1%. The mice in the TGN-020 group were injected intraperitoneally with a single dose of TGN-020 at 100 mg/kg, while the mice in the control group received a single intraperitoneal injection of the same volume of DMSO and phosphate-buffered saline (PBS). All invasive procedures, including intraperitoneal injections, were performed under sevoflurane anesthesia, using a concentration of

4% to ensure adequate anesthesia. Sevoflurane was supplied by Hengrui Pharmaceutical Company (Shanghai, China).

Cisterna magna injection

The function of the glymphatic system was assessed by observing the diffusion of Evans Blue in the brain. A microinjection syringe pump injected Evans Blue (1%, molecular weight: 960.81 g/mol, E2129, Sigma-Aldrich) into the cisterna magna. A 34-gauge needle was curved (45°) at 2 mm from the tip, with the beveled end facing laterally and attached to a 25 μL Hamilton microsyringe via plastic tubing. The microsyringe was controlled by a syringe pump. The posterior aspect of the skull at the level of the ears was identified, and the skin was incised approximately 1 cm along the midline using fine scissors. The occipital crest served as the reference point. The cisterna magna, obscured by the transparent dural membrane, was discernible as an inverted grey triangle after the neck muscles were retracted.

The needle tip was held parallel and punctured along the external surface of the occiput before being inserted into the cisterna magna. Evans Blue was injected into the cisterna magna at a total volume of 2 μL within 5 m. After injection, the needle was still held for 10 s, and the insertion site was sealed using super glue before the incision was suturing. All mice were then immediately awakened from anesthesia and allowed to move freely. Throughout the experiments, a thermostat was used to maintain the body temperature of the mice, and fluids were supplemented appropriately to ensure a stable physiological state. No mice died during the entire procedure.

Behavioral tests

After 8 h, we used the open field maze and Morris water maze tests to assess neurological function. In our experimental design, we implemented several measures to control environmental variables and ensure the reliability of behavioral changes in the mice. The experiments were conducted in a soundproof laboratory to minimize external noise disturbances. We monitored the temperature and humidity during the experiments to maintain appropriate conditions (e.g., temperature at 23 ± 2°C and humidity at 40–60%). The maze was cleaned before each experiment to eliminate any odors from previous tests that could affect the mice's behavior.

Open field maze test

We first used the open field maze (OF) test to assess locomotor and anxiety-related behaviors. The Ethovision XT software system (Noldus, Netherlands) was employed for behavioral analysis. Each mouse was placed in a 50 × 50 × 50 cm chamber and allowed to move freely for 20 m. The chamber was wiped with 75% ethanol before being used to remove any scent from previous mice, ensuring the ethanol evaporated completely before testing each mouse. Their trajectories were recorded to analyze total distance, movement speed, and time spent in the central area.

Morris water maze test

The Morris water maze (MWM) test was conducted immediately after the OF test to evaluate spatial memory. The water maze consisted of a circular pool with a diameter of 120 cm and a height of 50 cm, divided into four equal quadrants (I, II, III, and IV). Graphics of different shapes and colors were attached to the pool walls as markers. A blue curtain enclosed the pool to reduce external interference, and the light in the experimental room remained constant. The water depth was 30 cm, with the temperature maintained at 21 ± 1°C. A white dye was added to the water to obscure the platform and the pool's bottom. A 10 cm diameter platform was submerged 1 cm below the water's surface. The water maze experiment comprised two parts: familiarization and spatial exploration. The mice underwent a familiarization process over the last three days. On the day of the experiment, the mice were subjected to a TGN-020 injection (Fig. 1). They were placed in the water facing the wall and allowed to swim freely for 120 s to adapt to the environment. If a mouse did not find the platform within this time, it was gently guided to it and allowed to remain for 30 s. The time taken for the mice to find and board the platform was recorded as the escape latency. A spatial probe test followed, in which the water was removed from the pool. The mice were placed in the opposite quadrant and allowed to swim freely for 120 s, during which the number of platform crossings was recorded.

Blue dye analyses

At the end of all behavioral tests, mice were euthanized using an overdose of sevoflurane. The whole brain was carefully removed and examined to assess the intracranial distribution of the blue dye tracer and evaluate the effect of TGN-020 on the glymphatic system. A population-based average was created by combining images from all groups for both ventral and dorsal macroscopic photos, allowing for qualitative visual comparisons of the impact of TGN-020 on Evans Blue influx into the brain. The stained areas on the dorsal and ventral regions of the whole brain served as clear indicators of tracer influx. The quantitative area representing the amount of dye in the brain was analyzed, with dorsal and ventral images acquired using ImageJ (1.52p, National Institutes of Health, USA).

Immunohistochemistry for AQP4 expression

We chose 70 μm thick sections to balance tissue integrity and imaging quality. This thickness effectively preserves the three-dimensional features of cellular structures and allows for high-resolution imaging under the microscope. We focused on the selected cortex and observed the expression of AQP4. Brain slices were fixed with 4% paraformaldehyde for one day, followed by sucrose dehydration for one day. Antifreeze was prepared, and the frozen sections were washed with PBS three times for 5 m. They were then treated with 0.5% Triton-100 (PBS configuration) for 10 m, followed by three washes with PBS for 5 m each. Antigen retrieval was performed using sodium citrate buffer in a 98°C water bath for 15 m, followed by three washes with PBS for 5 m each. Incubation with anti-Aquaporin 4 antibody (1:500, Abcam) was conducted in a wet box at room temperature for 2 h. The secondary antibody, Alexa Fluor® 488 (1:1,000, Abcam), was then incubated in 1X PBS for three washes of 5 m each. Finally, we applied a DAPI anti-attenuation blocking solution to seal the slides and imaged them with a fluorescence microscope (FSX100, Olympus, Japan). We used image analysis software (ImageJ) to quantify the acquired fluorescence images. Specifically, we selected regions of interest and measured the fluorescence intensity. The expression intensity of AQP4 was quantified as the average fluorescence intensity of each region of interest, and the relative expression was derived after background correction.

Image pretreatment statistical analysis

Data are expressed as mean ± SD. Statistical comparisons between the experimental and control groups were performed using the one-way analysis of variance, depending on the outcome measure, with the statistical software GraphPad Prism, version 8.0.1 (GraphPad Software Inc., USA). A value of $p < 0.05$ was considered statistically significant.

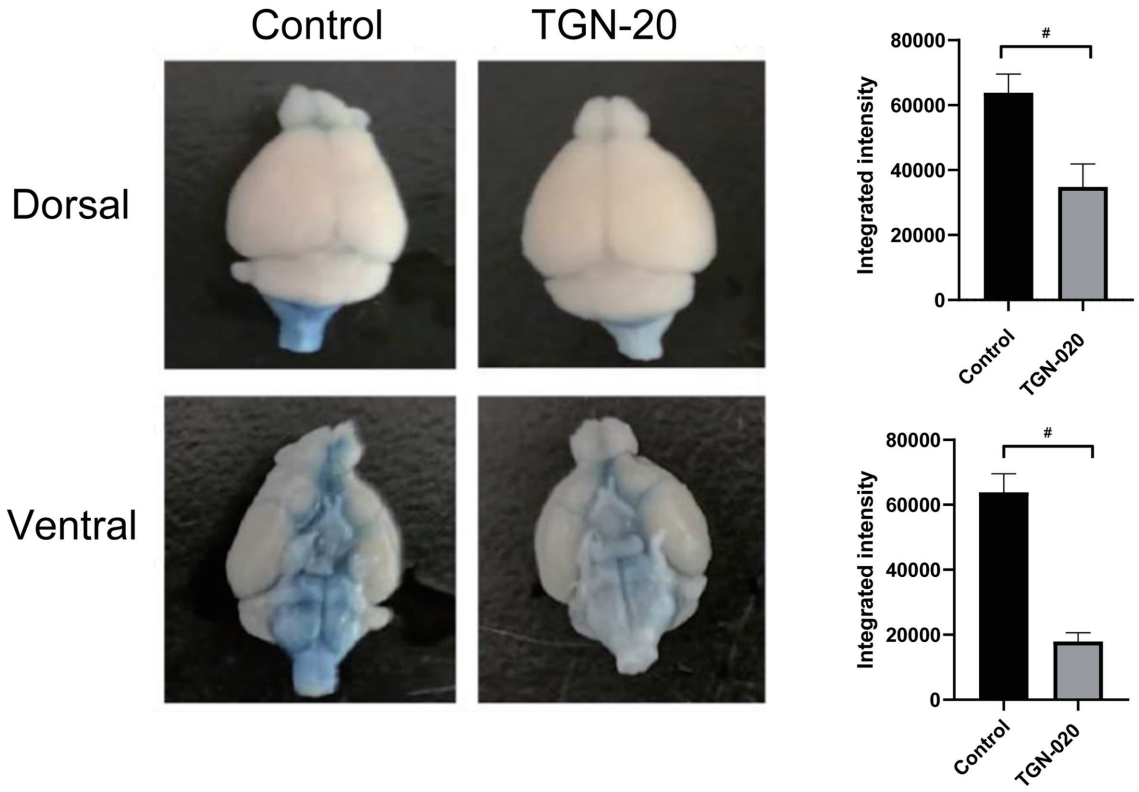

Fig. 2. TGN-020 significantly inhibited glymphatic function. Mice were injected intraperitoneally with TGN-020 (100 mg/kg), and the control group received the same volume of DMSO. Behavioral changes in both groups of mice were detected after 8 h, followed by observations of functional changes in the glymphatic system. The results showed that the area of Evans Blue on the brain surface of the TGN-020 group was significantly less than that of the control group. # $p < 0.05$. DMSO, dimethyl sulfoxide; TGN, trans-Golgi network; TGN-020, N-(1,3,4-thiadiazol-2-yl)-3-pyridinecarboxamide.

Results

A single dose of TGN-020(100 mg/kg) inhibits glymphatic influx into the brain for at least 8 h

Evans Blue was injected into the cisterna magna and flowed into the brain parenchyma through the glymphatic pathway to study the effect of TGN-020 on glymphatic function. We observed that the dye was distributed in the cerebellum, along the cerebral arterial circle and perivascular spaces, and in the olfactory bulb. The results showed less accumulation of Evans Blue on the brain surface in the TGN-020 group compared to the control group. The distribution of Evans Blue on the surface was significantly reduced by pretreating the mice with TGN-020 (Fig. 2). These results indicated that pretreatment with TGN-020 inhibits the influx ability of the glymphatic system, resulting in less influx into the cerebral perivascular space.

Pretreatment with TGN-020 resulted in no significant short-term behavioral changes

We used the OF test and the MWM test to assess neurological functions. Mice that received only TGN-020 pretreatment did not show significant changes in motor activity levels, exploratory behavior, or spatial memory. In the OF test, there were no significant alterations in the total distance traveled, average speed, or time spent in the center after pretreatment with TGN-020 (Fig. 3). The MWM test was also performed after the OF test to evaluate spatial memory. In the place navigation test, the escape latency of each mouse was recorded. Mice pretreatment with TGN-020 exhibited no significant changes in escape latency or the number of times they crossed the platform (Fig. 4). Notably, sighing breaths appeared immediately after injecting Evans Blue into the cisterna magna in all groups; however, this presentation lasted for only 5 m post-injection. No mouse died from respiratory insufficiency during the process.

A single dose of TGN-020 over a short period did not cause a significant change in AQP4 expression

The two groups' AQP4 expression intensity (the mean fluorescence intensity of AQP4) was comparable. No variations in AQP4 expression intensity were identified between the TGN-020 and control groups (Fig. 5).

Discussion

Recent studies have shown that pretreatment with TGN-020 significantly reduced the volume of cerebral edema associated with ischemic injury in a mouse model of cerebral ischemia.[8,9] It is suggested that the mechanism may involve effective modulation of glymphatic function. Our present results directly demonstrate that pretreatment with a single dose of TGN-020 can inhibit the influx related to glymphatic function, suggesting that after ischemic stroke, the direct inhibition of AQP4 function by TGN-020 significantly reduces the flow of cerebrospinal fluid via the inflow route of the glymphatic system to the brain parenchyma. However, the

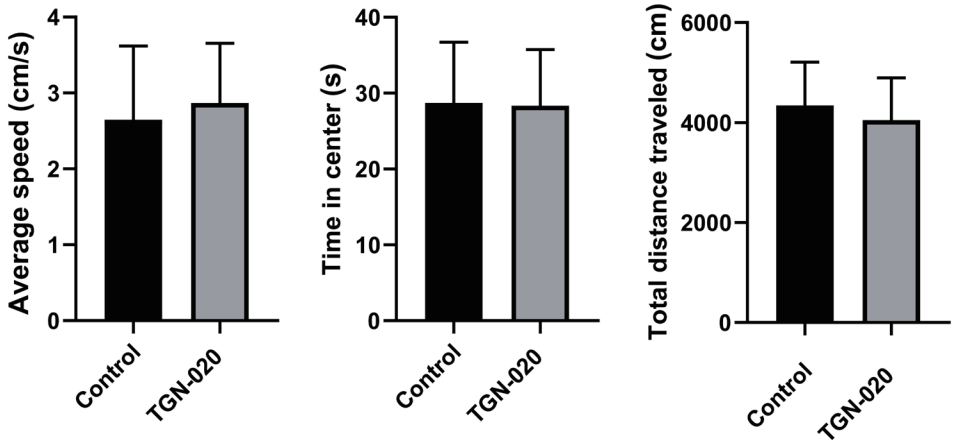

Fig. 3. Behavioral effects of TGN-020 on mice: results from open field experiments. The TGN group was injected intraperitoneally with TGN-020 (100 mg/kg), and the control group received the same volume of DMSO. Mice in both groups were allowed to move freely for 8 h, and behavioral studies were conducted afterward. Open-field results showed no significant differences between the two groups' total travel distance, mean velocity, or time spent in the center. DMSO, dimethyl sulfoxide; TGN, trans-Golgi network; TGN-020, N-(1,3,4-thiadiazol-2-yl)-3-pyridinecarboxamide.

role of AQP4 in regulating glymphatic function and cognition is only beginning to be understood. CSF flow depends on the expression and localization of AQP4. The possible mechanisms for this positive regulation could be related to the bidirectional transport function of AQP4, reduced water reabsorption, changes in osmotic pressure, or increased drainage of the glymphatic system.[6] Our study showed that no differences in AQP4 expression intensity were detected between the TGN-020 and control groups.

The fluid transport model of the glymphatic system indicates that the fluid influx pathway can penetrate deep into the brain parenchyma through the periarterial area, diffuse within the neurofibrillary network, where tissue fluid mixes with cerebrospinal fluid to exchange substances, and finally collect in the perivenous space to exit the brain.[13] Perivascular AQP4 is essential for the glymphatic clearance of harmful proteins and the dissipation of

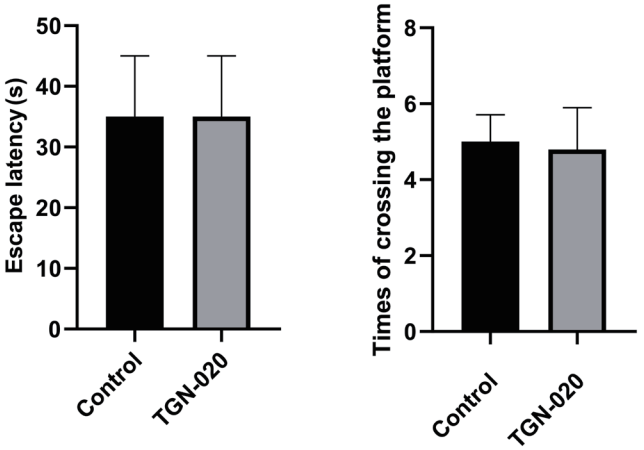

Fig. 4. Behavioral effects of TGN-020 on mice: results from water maze experiments. The TGN group was injected intraperitoneally with TGN-020 (100 mg/kg), and the control group received the same volume of DMSO. Mice in both groups were allowed to move freely for 8 h, and behavioral studies were conducted afterward. The water maze experiment results showed no significant difference between the two groups in escape latency and the number of platform crossings. DMSO, dimethyl sulfoxide; TGN, trans-Golgi network; TGN-020, N-(1,3,4-thiadiazol-2-yl)-3-pyridinecarboxamide.

vasogenic edema. However, the efflux of wastes or drugs from the brain has not been extensively studied due to the limitations of current technology, and research primarily focuses on the influx of the glymphatic system. This focus complicates efforts to accurately study how efflux rates change when TGN-020 inhibits influx. AQP4 is an important water channel protein in the brain, primarily located in the membranes of astrocytes, and is responsible for regulating intercellular water flow and fluid homeostasis.[14] TGN-020 inhibits the water channel activity of AQP4 by specifically binding to it, thereby reducing the flow of water between brain tissues. This inhibition may lead to restricted flow in the glymphatic system, affecting the efficiency of metabolic waste removal. AQP4 supports perivascular fluid and solute movement along the glymphatic system, providing a rapid route for water movement between the perivascular space and the glial syncytium. Our results also showed that pretreatment with a single dose of TGN-020 caused no short-term behavioral changes in healthy mice, suggesting that although a single dose of TGN-020 significantly inhibits glymphatic function, it does not cause substantial changes in brain function.

Invasive procedures such as puncture may trigger inflammation, which can cause dysfunction of the glymphatic system.[15] It has been shown that AQP4 exacerbates inflammation and apoptosis and that TGN-020 can attenuate the inflammatory response through AQP4 inhibition,[8] but the underlying mechanisms need further investigation. Some anesthetics, such as propofol, have antioxidant effects and can also affect the function of the glymphatic system,[16] which should not be underestimated in animal anesthesia. Additionally, the occurrence of sighing breaths after the injection of Evans blue into the cisterna magna in this study is likely due to transiently increased intracranial pressure or seizures, which may have caused central respiratory dysfunction.

Currently, some studies are developing chemicals that can safely and effectively modulate the glymphatic system, but no proven drugs have yet been developed. TGN-020 plays an active role in neuropathy by limiting the entry of fluid or medications into the brain and reducing cerebral edema. Therefore, TGN-020 may be a specific and potential therapeutic agent for neuropathological diseases, such as Parkinson's disease and cerebral stroke.[5,17–19] However, it also reduces the clearance of amyloid-β 40, a more

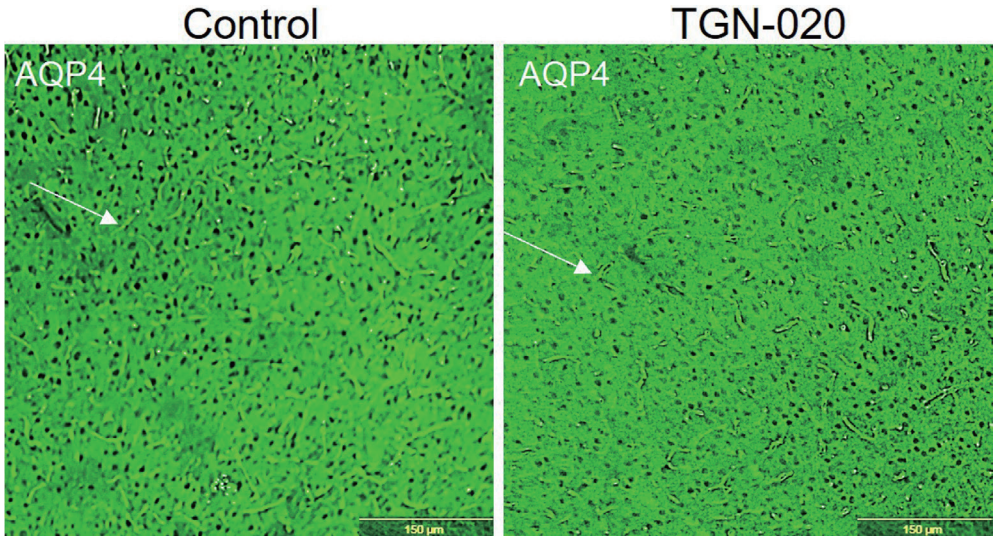

Fig. 5. Effect of TGN-020 on the expression of AQP4. The white arrows indicate the expression of AQP4. No differences in AQP4 expression intensity were found between the TGN-020 and control groups. AQP4, Aquaporin-4; TGN, trans-Golgi network; TGN-020, N-(1,3,4-thiadiazol-2-yl)-3-pyridinecarboxamide.

soluble isoform of amyloid-β, which may exacerbate Alzheimer's disease.[20] The systemic effects of TGN-020 on the glymphatic system in perioperative cognitive dysfunction remain to be investigated.[21] The influence of TGN-020 on the glymphatic pathway also depends on many factors. While acute inhibition of AQP4 shows no apparent short-term behavioral harmful effects, understanding the implications of long-term AQP4 inhibition is paramount, particularly in the context of chronic treatment protocols. Continued research is required to elucidate the long-term consequences of AQP4 inhibition, encompassing both protective and detrimental factors. This knowledge is essential for making informed decisions regarding the clinical application of AQP4 inhibitors and guiding the development of optimized therapeutic strategies that maximize patient benefits while minimizing potential risks.

The mechanism of action of TGN-020 and its effect on AQP4 is controversial.[22] Some studies suggest that TGN-020 may not completely inhibit the function of AQP4 but may instead affect its activity by altering the state of water channel proteins.[23] Although controversy remains regarding the specific inhibitory effect of TGN-020 on AQP4, our experimental results show that TGN-020 presents effects similar to those of other AQP4 inhibitors, particularly in terms of its impact on the glymphatic system. The action of TGN-020 may only partially depend on its specific inhibition of AQP4; it may also affect AQP4 function indirectly through other mechanisms or pathways, thereby influencing the existence of a glymphatic system. More studies are needed to verify this.TGN-020 is intended to selectively inhibit AQP4; nevertheless, like other pharmaceuticals, it may have off-target effects after administration. TGN-020 may influence not only AQP4 but also other types of aquaporins, including AQP1 and AQP5. These aquaporins are crucial in several tissues, such as the kidneys and lungs, and alterations in their function may disrupt systemic water regulation and electrolyte balance. TGN-020 may also interfere with further activities of astrocytes, such as neurotransmitter clearance and neuroprotection. This could result in glial cell malfunction, potentially impacting the overall neuronal environment and neuroplasticity. These topics will be comprehensively examined in future experiments.

In our study, the sample size was six mice per group, which was a consideration based on previous literature and actual experimental conditions. To enhance the reliability of our results, we will consider increasing the sample size in future experiments and conducting more comprehensive statistical analyses to improve the generalizability of our findings.

We utilized 10% DMSO as a solvent to generate the TGN-020 storage solution in our studies and subsequently diluted it to the target concentration of 2.5 mg/mL using PBS. We ultimately employed a low dosage of DMSO (≤0.1%), which is extensively documented in the literature and is typically regarded as having negligible effects on cell physiology under most experimental circumstances.[24] To evaluate the potential impact of DMSO on experimental outcomes, we conducted appropriate controls during the experimental design. Both the experimental and control groups were administered DMSO at an identical final dosage to mitigate any DMSO-induced background effects during result analysis.

We chose a normal mouse model to evaluate the inhibitory effect of TGN-020 on AQP4 independent of pathological conditions and to assess the safety of TGN-020 at a broad level. This choice allowed us to understand the mechanism of AQP4 inhibition at a fundamental level, laying the groundwork for subsequent applications in pathological models. Our main goal in selecting a normal mouse model was to systematically study the inhibitory effect of TGN-020 on AQP4 without pathological interference, enabling us to observe how TGN-020 affects AQP4 function in a physiological state. However, this choice also means that we cannot directly infer the effects the drug might have in pathological states (e.g., brain edema, neuroinflammation, etc.). Our future research could employ pathological models to validate the inhibitory effect of TGN-020 on AQP4 and its potential clinical applications, providing insights into the role of AQP4 in different pathological conditions.

This study employed a single dose of 100 mg/kg to observe its effects on the glymphatic system and AQP4 protein, based on relevant research literature. However, it did not explore in detail the impact of different doses on its action. In future experiments, we will examine the results of various doses to highlight the differences in their effects and to elucidate the dose dependency.

When the blood-brain barrier is intact, the uptake of Evans Blue is relatively low, making it difficult for the dye to penetrate. The extent of Evans Blue staining in the brain is directly related to the integrity of the blood-brain barrier and responds quickly to changes in the brain's glymphatic system. Evaluation of the glymphatic system using Evans Blue is relatively simple.[25] Combined with spectrophotometry, quantitative analysis of Evans Blue is straightforward and provides accurate results. However, Evans Blue is not a dedicated dye specifically for the glymphatic system; its accuracy is affected by blood-brain barrier permeability and may also be influenced by other pathological states (e.g., inflammation, tumors, etc.). Therefore, when assessing the function of the glymphatic system, it is necessary to consider various factors affecting Evans Blue clearance, including hemodynamics and the metabolic status of the tissue.

Future directions

TGN-020 is intended to selectively inhibit AQP4; nevertheless, like other pharmaceuticals, it may have off-target effects after administration. TGN-020 may influence not only AQP4 but also other types of water channels, including AQP1 and AQP5. These aquaporins are crucial in several tissues, such as the kidneys and lungs, and alterations in their function may disrupt systemic water regulation and electrolyte balance. TGN-020 may also affect other activities of astrocytes, such as neurotransmitter clearance and neuroprotection. This could lead to glial cell malfunction, potentially impacting the overall neuronal environment and neuroplasticity. These topics will be comprehensively examined in future experiments. Additionally, sympathetic excitation, blood pressure, vasoactive drugs, circulation capacity, and several other factors influence the function of the glymphatic vascular system. These related factors need to be investigated in future studies.

Conclusions

The administration of TGN-020, a selective Aquaporin-4 inhibitor, effectively impairs glymphatic function following a single dose. This blockade significantly reduces the clearance of interstitial solutes, underscoring the critical role of Aquaporin-4 in maintaining glymphatic activity. Furthermore, despite these physiological changes, our findings indicate that acute inhibition of Aquaporin-4 does not elicit noticeable short-term behavioral alterations in the subjects studied. These results suggest that while TGN-020 disrupts the glymphatic system, further investigations are needed to understand the long-term implications and potential compensatory mechanisms that may arise from prolonged inhibition of Aquaporin-4.

Acknowledgments

None.

Funding

This study was funded by the Jilin Provincial Department of Science and Technology (NO.YDZJ202401256ZYTS).

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Performing the behavioral experiments and writing the manuscript (CL), performing the experiments, preparing the manuscript (XR), assisting with parts of the experiments and the figures (TZ), and conception of the study (GZ, LL, PC). All authors read and approved the final manuscript.

Data sharing statement

The data supporting this study's findings are available upon reasonable request from the corresponding author. The datasets generated and analyzed during the current study are not publicly available due to concerns about participant confidentiality but can be made available for non-commercial research purposes. Please contact the corresponding author for further information on data access and sharing protocols.

Ethical statements

This study was approved by Jilin University's Ethics Committee. The Principles of Laboratory Animal Care (NIH publication no. 86-23 revised 1985), the applicable Animal Protection Law of the People's Republic of China, and the Regulations on the Management of Laboratory Animals were followed.

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