Effects of memantine treatment in a rat model of lacunar infarction

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Introduction

Lacunar stroke occurs when there is occlusion of small penetrating vessels providing blood to deep structures of the brain. They are believed to account for ~25% of all ischemic cerebral infarctions.

Memantine, an NMDA receptor antagonist commonly used as a neuroprotectant in Alzheimer's disease, has been shown to improve post-stroke outcome in rabbits[1], mice[2], and rats[3–5] by reducing cell death[3,4] and infarct volume[5]. To date, no work has been done to assess the potential efficacy of *pre*-stroke administration of the drug in lacunar stroke model. *In vitro* studies have shown memantine works in part by protecting neurons against excitotoxicity[6], the major cause of cell death during stroke. Therefore, further *in vivo* evaluation is warranted.

This study was performed to evaluate the effects of pre-treatment with memantine prior to lacunar infarction in a rat model. It was hypothesized that this method would result in a decrease of lesion size and affect the inflammatory response.

Methods

Drug administration

Memantine (or saline vehicle) was administered via intraperitoneal injection (20 mg/kg) 30 mins pre-stroke.

Surgical Stroke

Rats were anesthetized and placed in a stereotaxic apparatus under isoflurane anesthesia (2%). Endothelin-1 (400 pmol/in 0.5 µl) was be injected (0.5 µl/min) using a microsyringe at AP +0.7; ML +3.8; DV -0.7.

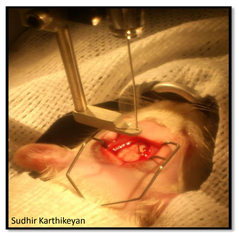

Figure 1. Following anesthesia, the scalp was incised and retracted, and a small burr hold was drilled at each injection location.

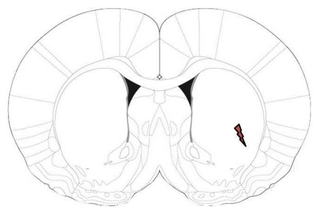

Figure 2. Schematic diagram of a brain section indicating site of the endothelin-1 microinjection location. ET-1 acts as a vasoconstrictor, causing temporary constriction of blood flow to all vasculature in that area of the brain.

Blood Sampling

Following induction of stroke, blood was sampled from the saphenous vein. Samples were clotted for 1 hour at room temperature, then centrifuged. The serum was collected and frozen at -80˚ C. Samples were analyzed by HPLC to determine serum memantine levels, determined to be 2.846 ± 0.511 nM.

Figure 3. Blood was sampled from the saphenous vein, clotted, centrifuged, and the serum was separated for analysis of drug levels.

Results

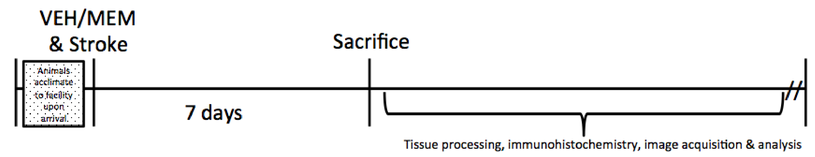

Figure 5. Experimental Timeline. Following animal acclimation to the facility upon arrival, adult male Sprague Dawley rats received a single intraperitoneal dose of memantine (or vehicle control). Thirty minutes later, they were subjected to surgically induced lacunar stroke using intracerebral injections of endothelin-1. One week later, animals were sacrificed by transcardial perfusion and brains were processed for histological analyses.

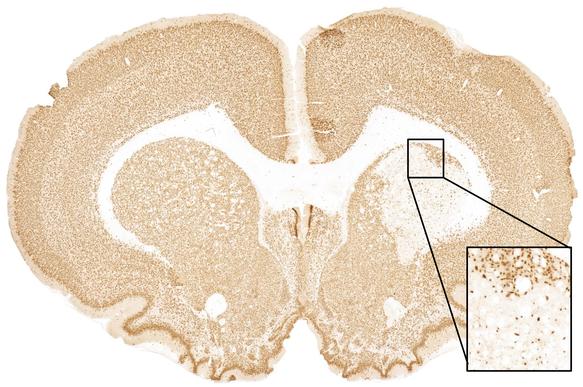

Figure 4. NeuN is a commonly used label of neurons. The border of NeuN+ areas were traced using ImageJ and multiplied by distance between sections to obtain volume of injury. The edge of the infarct was defined as the border of NeuN+ cells as shown in the enlarged box. Several NeuN+ cells were visible within that defined infarct, which were also counted.

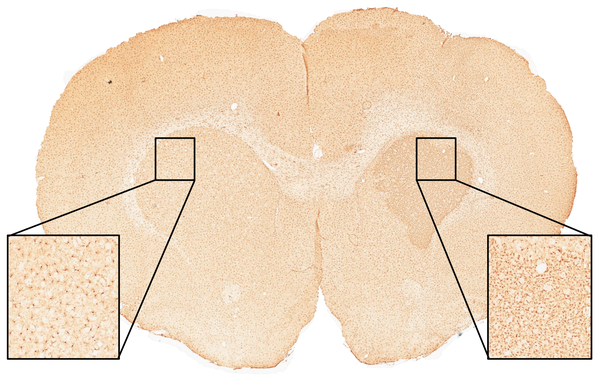

Figure 5. Iba1 is a marker of microglia, the major form of defense in the central nervous system. Iba1 reactivity was compared between groups by determining percent area of damaged striatum covered by Iba1, normalized against the uninjured hemisphere, using binarized images. In the left enlarged box, ramified resting microglia can be seen in the uninjured hemisphere. In the right enlarged box, activated microglia can be seen to culminate in dense clusters throughout the stroke area.

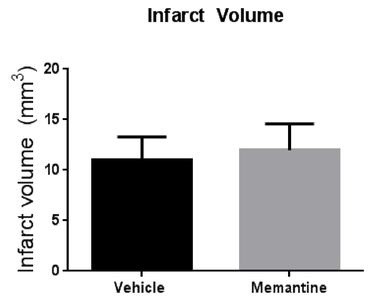

Figure 6. VEH treatment (n=12) resulted in a mean infarct volume of 10.98 ± 2.31 mm^3. MEM treatment (n=11) resulted in a mean infarct volume of 11.98 ± 2.59 mm^3. Using the independent samples T-test, we obtain p=0.777, indicating there was no significant difference between groups.

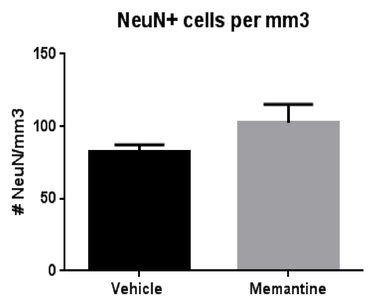

Figure 7. VEH treatment (n=12) resulted in a mean of 82.735 ± 5.105 NeuN+ cells per mm^3 of injury. MEM treatment (n=11) resulted in a mean of 100.413 ± 11.661 NeuN+ cells. Using the independent samples T-test, we obtain p=0.167, indicating there was no significant difference between groups.

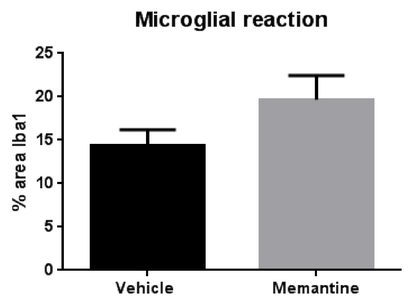

Figure 8. VEH treatment (n=4) resulted in a mean percent Iba1 area of 14.354 ± 1.835 %. MEM treatment (n=4) resulted in a mean percent Iba1 area of 19.664 ± 2.779 %. Using the independent samples T-test, we obtain p=0.162, indicating there was no significant difference between groups.

Methods (continued)

Sacrifice and tissue collection

Animals were transcardially perfused with heparanized saline followed by 4% paraformaldehyde (PFA). The brains were then removed, frozen and stored at -80˚ C, and were then sectioned (20 um) using a cryostat. Every 10th section was used for analysis.

Immunohistochemistry

Sections were subjected to heat induced epitope retrieval in 95˚ C citric acid for 20 minutes, then cooled and incubated with mouse anti-NeuN (1:250) or rabbit anti-Iba1 (1:500) antibodies 4˚ C overnight. The next day, they were incubated with secondary antibodies, ABC, and diaminobenzidine (DAB) for visualization.

Statistics

Infarct volume, NeuN+ cells in damage core, and Iba1 reactivity were compared between groups using independent samples T-tests (SPSS).

Conclusions

Infarct volume was not affected

Infarct volumes in rats having received memantine via intraperitoneal injection pre-stroke were not statistically different from those of rats having received saline vehicle.

NeuN cells in the infarct core

Statistical analysis of the amount of NeuN+ cells in the infarct area concluded that memantine did not result in a significant difference in number. However, the memantine group did demonstrate a slightly greater average of surviving cells. This difference may be further established using a larger sample size.

Microglial reaction

Analysis of microglia, the major form of defense in the central nervous system, was performed to determine whether MEM affects the immune reaction. The percent Iba1-encompassed area was found to be slightly larger in the memantine group, although not statistically significant. As each group represented a very small sample size (n=4), the difference may prove to be significant pending further analysis of remaining subjects.

Future Directions

This work has been integral in providing some baseline data on the nature of how the brain responds to lacunar injury in the first 7 days, and how memantine may act on these mechanisms. While not statistically significant, the promising NeuN+ infarct core neurons and the interesting Iba1 result have lead to further investigations into apoptosis in the infarct core (by caspase investigation) and activated microglia (ED-1), which are presently ongoing.

Acknowledgements

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