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, mice, and rats by reducing cell death and infarct volume. 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, 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

Drugs administration
Memantine (or saline vehicle) was administered via intraperitoneal injection (20 mg/kg) 50 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.

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.

Sacrifice and tissue collection
Animals were transcardially perfused with heparinized saline followed by 4% paraformaldehyde (PFA). The brains were then removed, frozen and stored at -80°C, and were then sectioned (20 μm) 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).

Results

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-encapsulated 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 apotosis in the infarct core (by caspase investigation) and activated microglia (ED-1), which are presently ongoing.

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References

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