Delayed Varenicline Administration Reduces Inflammation and Improves Forelimb Use Following Experimental Stroke

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Background: Pharmacological activation of the cholinergic anti-inflammatory pathway (CAP), specifically by activating α7 nicotinic acetylcholine receptors, has been shown to confer short-term improvements in outcome. Most studies have investigated administration within 24 hours of stroke, and few have investigated drugs approved for use in human patients. We investigated whether delayed administration of varenicline, a high-affinity agonist at α7 nicotinic receptors and an established therapy for nicotine addiction, decreased brain inflammation and improved functional performance in a mouse model of experimental stroke. *Methods:* CSF-1R-EGFP (MacGreen) mice were subjected to transient middle cerebral artery occlusion and administered varenicline (2.5 mg/kg/d for 7 days) or saline (n = 10 per group) 3 days after stroke. Forelimb asymmetry was assessed in the Cylinder test every 2 days after surgery, and structural lesions were quantified at day 10. Enhanced green fluorescent protein (EGFP) and growth associated protein 43 (GAP43) immunohistochemistry were used to evaluate the effect of varenicline on inflammation and axonal regeneration, respectively. *Results:* Varenicline-treated animals showed a significant increase in impaired forelimb use compared with saline-treated animals 10 days after stroke. Varenicline treatment was associated with reduced EGFP expression and increased GAP43 expression in the striatum of MacGreen mice. *Conclusion:* Our results show that delayed administration of varenicline promotes recovery of function following experimental stroke. Motor function improvements were accompanied by decreased brain inflammation and increased axonal regeneration in nonpenumbral areas. These results suggest that the administration of an exogenous nicotinic agonist in the subacute phase following stroke may be a viable therapeutic strategy for stroke patients. **Key Words:** Cholinergic anti-inflammatory pathway—Functional recovery—Ischemic stroke—MacGreen mice—Varenicline—GAP43.

Introduction

Brain injuries, such as stroke, are associated with profound activation of inflammatory pathways. While inflammation is important in resolving injuries, prolonged inflammation hinders tissue repair and, ultimately, patient prognosis.[1-3]

Immune responses and inflammation are regulated in part by neural mechanisms.[4] The parasympathetic nervous system can inhibit cytokine release and prevent tissue injury via an efferent neural signaling pathway termed the cholinergic anti-inflammatory pathway (CAP).[4,5] Stimulation of the vagus nerve attenuates production of TNFα and other proinflammatory cytokines from macrophages in the spleen through a mechanism dependent on α7 nicotinic acetylcholine receptors (nAChRs).[4,6-8]

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It remains unclear why this homeostatic mechanism fails to limit the extent of brain inflammation that occurs after stroke. Clinically, low heart-rate variability and chronic inflammation correlate with poor neurological outcomes in stroke patients.[9-13] This suggests that the parasympathetic nervous system tone is reduced following stroke and that this autonomic imbalance leads to suboptimal activation of the CAP, increased inflammation, and inhibition of recovery and repair processes.

Autonomic regulation via stimulation of the vagus nerve has been shown to be a viable strategy for targeting diseases with an inflammatory component such as rheumatoid arthritis and hemorrhagic shock.[14,15] Vagal nerve stimulation within 30 minutes of injury has also been shown to reduce brain damage in experimental models of stroke.[16-18] More recently, direct activation of α7 nAChRs has been investigated as a less invasive and more applicable therapeutic strategy to reduce brain inflammation and ultimately promote functional recovery after stroke. Both α7 nAChR agonists and allosteric modulators display neuroprotective and anti-inflammatory effects in rodent models of focal[19,20] and global ischemia and traumatic brain injury.[21] To date, these studies have initiated administration within 6 hours of injury, and none have demonstrated functional improvement beyond 7 days poststroke.[20] It is not known whether activation of nAChRs in the subacute phase (>24 hours) after stroke can also modulate brain inflammation and promote recovery of function.

The CSF-1R-EGFP (MacGreen) mouse has been show previously to provide a model system in which to investigate brain inflammation following experimental stroke. The aim of this study was to investigate whether delayed administration of the α7 nAChR agonist varenicline reduced brain inflammation and improved motor function in CSF-1R-EGFP mice.

Materials and Methods

Ethical Statement

All animal work was carried out in accordance with the Animal Welfare act 1999 and was preapproved by the University of Auckland Animal Ethics Committee (Approval number: R842).

Animals

Founder CSF-1R-EGFP (MacGreen) mice were gifted by the Queensland Brain Institute, University of Queensland, Australia. Generation of the strain has been described elsewhere.[22,23] The MacGreen colony was maintained as homozygotes and all offspring were positive for the transgene. The ongoing presence of the transgene within the colony was confirmed every 2 months from tail biopsies using standard polymerase chain reaction methods. Tail tipping was performed under isofluorane anesthesia. DNA extraction from tail biopsies was performed using a REDExtract-N-Amp Tissue polymerase chain reaction kit (Sigma-Aldrich, Auckland, NZ) using forward 5′-CTGGTCGAGCTGGACGGCGACG-3′ and reverse 5′-CACGAACTCCAGCAGGACCATG-3′ primers, producing a 650 base pair product.

All animals were housed in a single-sex cage and supplied with standard rodent chow and water available ad libitum. Housing was maintained at 20°C with a 12-hour day and night cycle. CSF-1R-EGFP "MacGreen" mice (25-30 g, approximately 10-12 weeks) were assigned to treatment groups (varenicline or saline, n = 10 per group) using a randomization table generated by Excel (Microsoft, Washington, USA). Body weight, neurological score, and exploratory forelimb use in the Cylinder test was recorded at baseline and for 10 days following stroke.

Cerebral Ischemia

Monofilament occlusion of the middle cerebral artery (MCA) was performed in adult male MacGreen mice (25–30 g, approximately 10-12 weeks) as previously described.[24] Briefly, the right common carotid (CCA), external carotid (ECA), and internal carotid (ICA) arteries and their branches were exposed through a midline cervical incision. A 6-0 silk suture was tied around the CCA proximal to the bifurcation of the ECA and ICA, and a second suture was tied around the ECA distal to the superior thyroid artery. The super thyroid artery and occipital artery were occluded by electrocoagulation. An 8-0 silicone-coated monofilament (200 μm diameter) was introduced into the CCA and advanced 10 mm distal to the carotid bifurcation, occluding the origin of the MCA. Mice were subjected to occlusion of the MCA in their dominant hemisphere (based on baseline functional performance) for 45 minutes. Core temperature was maintained at 36.5°C ± .5°C throughout the course of the surgery by means of a rectal probe connected to a homoeothermic heating blanket (Harvard Apparatus).

At the end of the occlusion period, the monofilament was removed and the surgical site sutured closed. Animals were recovered in a humidified incubator until freely moving and then returned to their home cages.

Drug Delivery

Due to its short half-life in mice (1.4 hours),[25] varenicline was administered by a mini–osmotic pump to maintain a steady plasma concentration. The dose of the varenicline used in this study was based on previous studies in mice.[26,27]

Varenicline tartrate (YES Pharma, Ltd., Beit Shemesh, Israel) was dissolved in .9% saline with a few drops of .4 M NaOH added to aid dissolution. The quantity of drug required to deliver 2.5 mg/kg/d for 10 days was calculated from the individual body weight of each mouse. Mini–osmotic pumps (1007D; Alzer Osmotic Pumps, Cupertino, CA, USA) were filled with saline (n-10) or