Delayed Administration of the Glucagon-Like Peptide 1 Analog Liraglutide Promoting Angiogenesis after Focal Cerebral Ischemia in Mice

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Background: Glucagon-like peptide 1 (GLP-1) analogs administered before or after cerebral ischemia have been shown to provide neuroprotection. Here, we explored whether delayed administration of a GLP-1 analog, liraglutide, could improve long-term functional recovery and promote angiogenesis after stroke. *Materials and Methods:* In the present study, mice were established as a focal cerebral cortical ischemia model and were intraperitoneally administered liraglutide or normal saline (NS) daily for 14 consecutive days, starting 1 day after cerebral ischemia. The neurological deficits were evaluated using rotarod test. The microvessel density (MVD) and endothelial cell (EC) proliferation were assessed by immunohistochemical staining. The expression of vascular endothelial growth factor (VEGF) was assessed by Western blot analysis. *Results:* Liraglutide significantly reduced infarct volume and improved the rotarod test scores, compared with mice treated with NS. Liraglutide also greatly increased the MVD and EC proliferation and simultaneously upregulated the expression of VEGF in the cerebral ischemic area. *Conclusions:* These results demonstrated that liraglutide promoted angiogenesis and long-term recovery of cerebral ischemia through increasing the expression of VEGF. **Key Words:** Cerebral ischemia—liraglutide—glucagon-like peptide 1 (GLP-1)—angiogenesis—vascular endothelial growth factor (VEGF).

Introduction

Stroke is one of the chief causes of death and severe long-term disability worldwide, which causes a huge public financial burden. About 80% of patients with stroke are ischemic.[1] Current treatment options believe that intravenous thrombolysis has been shown to be the only effective intervention for acute ischemic stroke.[2] However, this treatment benefits less than 10% of patients with ischemic stroke because it has a narrow therapeutic time window of 3.0-4.5 hours and the risk of intracranial hemorrhagic stroke.[3] So, more and more attention has been drawn to develop novel strategies, which can be executed outside of this therapeutic window. Brain repair after stroke requires various processes, including neurogenesis, angiogenesis, and synaptogenesis. Angiogenesis has emerged as a novel promising therapeutic target for stroke.[4] The formation of new blood vessels by angiogenesis improves tissue microperfusion in the

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peri-infarction region after ischemic cerebral injury.[5] Glucagon-like peptide 1 (GLP-1) is an incretin hormone that is secreted from the L-cells of the small intestine, which plays a crucial role in blood glucose control.[6] GLP-1 and its analogs can stimulate glucose-dependent insulin secretion from pancreatic beta cell and inhibit the secretion of glucagon.[7,8] It rarely causes hypoglycemia. Therefore, GLP-1 analogs have been approved for clinical treatment in type 2 diabetes mellitus. GLP-1 exerts its effects by binding to GLP-1 receptor (GLP-1R), which is found to be expressed not only in the pancreas but also in extrapancreatic organs, including the brain, lung, kidney, and atrial cardiomyocytes.[9] It implies that GLP-1R activation plays an extensive range of extrapancreatic functions. Liraglutide has been considered a true long-lasting GLP-1 analog, sharing 97% sequence identity with human GLP-1, which is widely used for the treatment of type 2 diabetes.[10] Owing to their small molecule size, both GLP-1 and liraglutide are able to cross the blood–brain barrier in animal studies after peripheral administration.[11] Besides its antidiabetic effects, previous studies have reported that liraglutide provide neuroprotection against the damage of cerebral ischemia.[12] A previous study has found that liraglutide, administered intraperitoneally after stroke, reduced infarct volume and provided neuroprotection against cerebral ischemia–reperfusion injury. Based on these studies, we assess whether delayed treatment with liraglutide (1 day after stroke) improves long-term functional outcome and promotes angiogenesis after stroke, as well as explore the possible mechanisms.

Materials and Methods

Animals

Adult male CD-1 mice weighing 25-30 g at the beginning of the experiment were purchased from the Vital River, Beijing, China. All animals were kept at a controlled temperature and humidity in each separate cage, under a 12-hour light–dark cycle. The animals were fed ad libitum during the experiment. They were allowed to acclimatize to the new surrounding for at least 3 days before being used in the experiments.

Cerebral Ischemia

All experimental procedures were approved by the Institutional Animal Care and the Animal Care and Management Committee of Second Hospital of Hebei Medical University. A standard model of permanent distal middle cerebral artery occlusion (dMCAO) was performed according to a previously published protocol.[13] All animals were anesthetized by intraperitoneal injection of Avertin (2.4 mg/10g, Sigma-Aldrich, St. Louis, MO, USA). During the operation, body temperature was monitored and maintained at 37°C with a heating pad until the animals woke from anesthesia. Firstly, the right common carotid artery was isolated and ligated by a 6-0 silk suture. Then, another incision between the right ear and eye exposed the skull, and a craniotomy was conducted using a high-speed dental drill. Finally, under a microscope, the right middle cerebral artery (MCA) distal to the striatal branch was exposed and permanently coagulated with a coagulator (Bovie Aaron Medical, USA). The sham group underwent similar surgical procedures except for the electrocoagulation of the MCA. The mice that had intracerebral, subarachnoid hemorrhage or died after surgery were excluded and had early euthanasia end points.

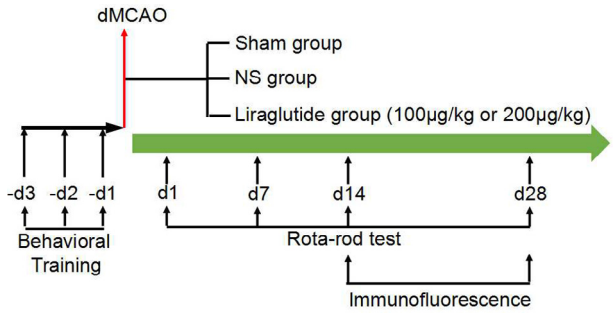

Figure 1. *Schematic diagram of the experimental design. All mice were assigned to 3 groups: (1) sham group, (2) NS group, and (3) liraglutide group (100 or 200 μg/kg). An equal volume of NS or liraglutide was intraperitoneally administered to the corresponding group for 14 consecutive days as early as 1 day postoperatively. All mice were trained for 3 days before surgery on the rotarod test. The rotarod test was performed on d1, d7, d14, and d28 after cerebral ischemia. Immunofluorescence was performed on d14 and d28. Abbreviations: d, day; dMCAO, distal middle cerebral artery occlusion; NS, normal saline.*

Groups and Drug Administration

The detail experimental procedure is shown in Figure 1. In the first set of studies, the mice were randomly assigned to 4 groups: sham group, normal saline (NS) group, and 2 liraglutide groups (100 and 200 μg/kg). The dosage regimen of liraglutide was determined based on a previous study.[14] The day of surgery was defined as day 0. Liraglutide (GL Biochem Ltd, Shanghai, China) was dissolved in NS and administered to the liraglutide group at 100 or 200 μg/kg for 14 consecutive days through intraperitoneal injection as early as 1 day postoperatively. The sham group and the NS group received an equal volume of NS in the same way. These mice were used for the rotarod test, and the optimal dose of liraglutide was determined in the following experiments.

In the second set of studies, mice were randomly assigned to 3 groups: sham group, NS group, and liraglutide group (200 μg/kg). These mice were administered with an intraperitoneal injection of 5-bromo-2′-deoxyuridine (BrdU, 50 mg/kg; Solarbio, Beijing, China) once daily for 14 consecutive days as early as 1 day postoperatively. At 7, 14, and 28 days after stroke as the experimental design, the mice were killed at each time point, and the