SUPPLEMENTAL MATERIALS

Methods and Materials

Immunohistochemical Staining and Semi-quantitative Determination

Liposomal delivery of angiogenic peptides and therapeutic effectiveness were also immunohistologically studied by semi-quantitatively determining vascular density using von Willebrand factor-positive staining and neuroprotection using glial fibrillary acidic protein (GFAP)-positive staining (n=12/group, respectively). Animals treated with and without liposomal angiogenic peptides were sacrificed eight days post-surgery. The brains were fixed with 10% formalin for 48 hours, washed, and paraffin-embedded. The coronal slices of the brains were sectioned (6 μm in thickness), de-paraffinized, and rehydrated. After heat treatment, the tissue specimens were incubated overnight (4 ^{o}C) with the primary antibody against von Willebrand factor (DAKO; 1:500) as a marker for endothelial phenotype and GFAP (abcam; 1:3000) as a marker for astrocyte activation. After incubating with the primary antibodies, the sections were conjugated with biotin-labeled secondary antibodies, which were later detected with 3-amino-9-ethylcarbazole chromogen (Dako). The immuno-stained tissues were then photographed with a camera connected to a microscope.

The total number of von Willebrand factor-positive vessels present in the ischemic border and GFAP-positive cells present in the ischemic hemisphere were counted. Six fields were randomly chosen from the areas of interest. The means from the six fields are presented.

RNA Isolation and RT-PCR Analysis

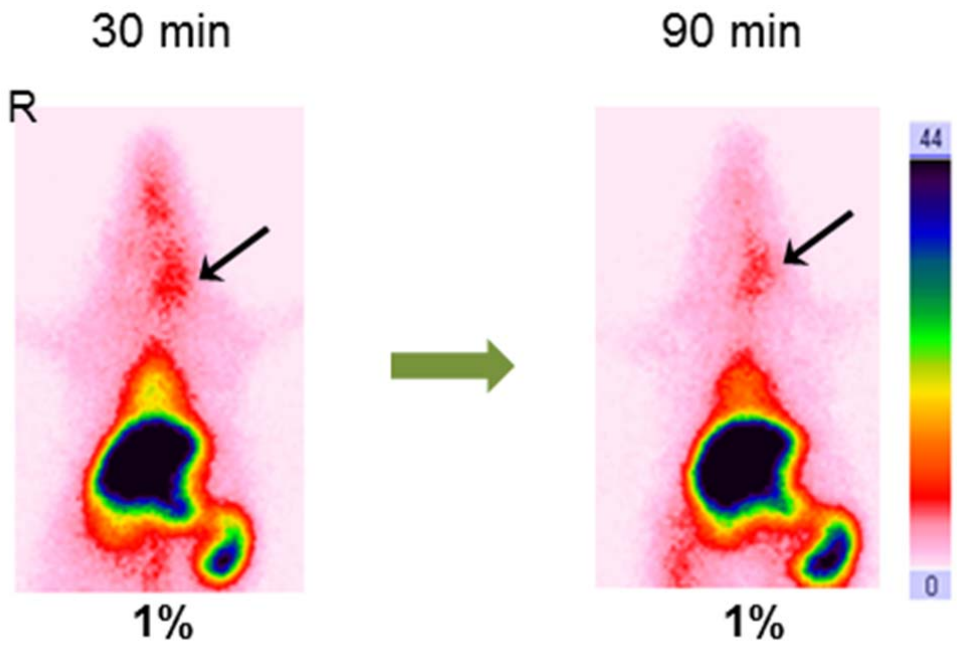
The total RNA (n=4/groups) was isolated from the ipsilateral cortex using RNeasy mini kits (Qiagen). Briefly, the tissues from the ipsilateral cortex of the brain were homogenized using a dounce homogenizer in RLT lysis buffer, and RNA was extracted using an elution

volume of 40 μl. Complementary DNA was reverse transcribed from the purified RNA (1 μg) using RNA to cDNA EcoDry™ Premix (Life technology) in a total volume of 20 μl (Clontech). The reaction condition was 42 °C for 60 minutes, followed by 70 °C for 10 minutes using the ABI 9700 thermal PCR cycler (Applied Biosystems). The RT reactions were subjected to quantitative PCR amplification. The complementary DNA products (5 μl) were amplified with SYBR Premix Ex Taq II (Takara Bio). The cycling parameters were: one cycle of 95 °C for 10 minutes, followed by 45 cycles of 95 °C for 15 seconds, and 60 °C for 1 minute on a StepOne Plus system (Applied Biosystems). All reactions were performed in triplicate. Absolute expression levels were calculated after normalization to β-actin. The levels of mRNA were expressed as the ratio of negative control without ischemia.

The RT-PCR products were further visualized after electrophoresis migration in a 2% ethidium bromide-stained agarose gel. The bands were viewed under UV light.

Gamma Imaging

Scintigraphy was carried out after administration of liposomes labeled with ^{99m}Tc HMPAO. ^{99m}Tc HMPAO (7.4 MBq) was administered via the tail vein, and images were captured at 30 and 90 minutes post-treatment using a gamma image acquisition system.



Supplemental Figure 1. Radionuclide images of rats following 40 minutes of ischemia show cerebral retention of the ^{99m}Tc HMPAO-liposome in the ipsilateral hemisphere of the brain. Rats undergoing middle cerebral artery occlusion received ^{99m}Tc HMPAO-liposomes intra-arterially. SPECT-CT imaging was obtained at 30 and 90 minutes post-injection. Arrows indicate cerebral uptake of ^{99m}Tc liposomes.