SUPPLEMENTARY MATERIAL

Supplementary Methods

Myeloperoxidase (MPO) activity assay

To measure the MPO activity, contralateral (control) and ischemic (infarcted and surrounding areas) regions were homogenized and extracted in 1% cetyltrimethylammonium bromide (Sigma-Aldrich) in 50 mM KPO_4 buffer, pH 7.0. The resultant suspensions were sonicated for 30 s with 3 cycles of freeze-thaw in liquid nitrogen. Subsequently, the suspensions were centrifuged at 16,000 X g for 15 min and supernatant fractions were used for measurement of MPO activity and protein estimation. 100 µl of tetramethylbenzidine (TMB) solution (Sigma-Aldrich) was added to 40 µl of supernatant in a 96-well plate in duplicates. The reaction was stopped with 100 µl 1N HCl after 10 minutes and read at 450 nm. MPO activity was calculated using an MPO standard (Sigma-Aldrich) and the resultant activity was normalized as MPO units/mg of protein.

Immunohistochemical analysis

Immunostaining of neutrophils and macrophages was slightly modified and done as described.[1] Briefly, 9.0 µm coronal sections of fresh frozen tissue were incubated with blocking reagent followed by primary antibody (rat anti-mouse neutrophil marker (NIMP) or rat anti-mouse Mac-3) or rat Ig (control) in the presence of 5% goat serum overnight at 4°C followed by biotin-conjugated goat anti-rat Ig, avidin-biotin complex, and 3,3′-diaminobenzidine as substrate. Slides were counterstained with hematoxylin, dehydrated, and examined under a light microscope (Zeiss). Immunoreactive cells (brown staining) were counted in five different cerebral regions (3 within parietal cortex and 2 within basal ganglia) across different stereotactic levels. The ratio of immunoreactive cells per total number of cells in the defined infarcted area was used to calculate

the fraction of immuno-positive cells. A mean was calculated from the five different cerebral regions to represent each section. Nine coronal sections, separated by 100 μm from the frontal pole, per mouse were analyzed.

Western Blot Analysis

Brain cortical tissue was collected from the infarcted and surrounding areas. Cytosolic and nuclear extracts were prepared as described.[2] Samples of nuclear and cytoplasmic fractions containing equal amounts of protein (40 μg) were separated in 10 % SDS-polyacrylamide gel electrophoresis. Proteins were transferred onto PVDF membrane (Hybond- P) and incubated overnight at 4°C with specific primary rabbit polyclonal antibody against NF-κB p65 (1:2000, Rockland, PA, USA), or a goat polyclonal antibody against COX-2 (1:200, Santa Cruz, USA) followed by appropriate secondary antibodies conjugated to horseradish peroxidase (HRP). Proteins recognized by the antibody were visualized by enhanced chemiluminescence Femto kit (Thermo Scientific) according to manufacturer instructions. All blots were stripped and re-incubated with primary antibody specific to β-actin (Sigma) as a loading control. Intensity of the bands was measured by densitometry and quantified using NIH-Image J software.

ELISA assay for TNF-α, IL-1β, IL-6

To prepare homogenates brain cortical tissue was collected from the infarcted and surrounding areas and lysed in tissue lysis buffer (20 mM Tris-Cl, pH 8.0, 10 mM NaCl, 2% Triton X-100). Supernatants from brain homogenates were used for determination of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) with commercially available mouse ELISA kits (R&D Systems) according to the manufacturer's instructions.

TLR4 inhibitor

TLR4 specific inhibitor CLI-095 (Invivogen, San Diego, USA) was prepared according to the manufacturer's instructions. Mice were injected intravenously with CLI-095 (1mg/Kg) or vehicle 30 minutes prior to 60 min ischemia/ 23 h reperfusion injury. Prior to sacrifice mice were evaluated for neurological deficits as a functional outcome as described above.

References

1. Breckwoldt MO, Chen JW, Stangenberg L, Aikawa E, Rodriguez E, Qiu S et al. Tracking the inflammatory response in stroke in vivo by sensing the enzyme myeloperoxidase. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;105:18584-18589
2. Cardenas A, Moro MA, Hurtado O, Leza JC, Lorenzo P, Castrillo A et al. Implication of glutamate in the expression of inducible nitric oxide synthase after oxygen and glucose deprivation in rat forebrain slices. *Journal of neurochemistry*. 2000;74:2041-2048

Supplementary Tables

Groups	Ischemia LDF (%)	Reperfusion LDF (%)
WT	13 ± 2	79 ± 5
$EDA^{+/+}$	14 ± 1	82 ± 4
$EDA^{+/wt}$	16 ± 3	83 ± 7
WT (Treated with TLR4 inhibitor)	16 ± 2	80 ± 7
$EDA^{+/+}$ (Treated with TLR4 inhibitor)	16 ± 3	89 ± 8

Table 1. Laser Doppler Flowmetry (LDF) was similar among groups during and after ischemia. Values are expressed as mean ± SEM. N= 8-10 mice/group

Variables	WT		$EDA^{+/+}$	
	Before	After	Before	After
pH	7. 34 ± 0.05	7.28 ± 0.04	7.32 ± 0.05	7.26 ± 0.06
pO2, mmHg	129 ± 8	138 ± 9	133 ± 11	125 ± 20
pCO2, mmHg	34 ± 11	42 ± 6	37 ± 9	43 ± 11
MABP	71 ± 7	65 ± 6	60 ± 7	62 ± 5
Body temperature	35.9 ± 0.5	36.3 ± 0.2	36.5 ± 0.3	36.7 ± 0.4

Table 2. Physiological parameters. 50 μl of blood was withdrawn before and after ischemia for blood gases determination. Values are expressed as mean ± SD. Before: before ischemia; After: 60 min after ischemia; MABP: Mean Arterial Blood Pressure under anesthetized conditions. Physiological parameters were similar among groups during the procedure. N= 4 mice/group.

Supplementary Figure

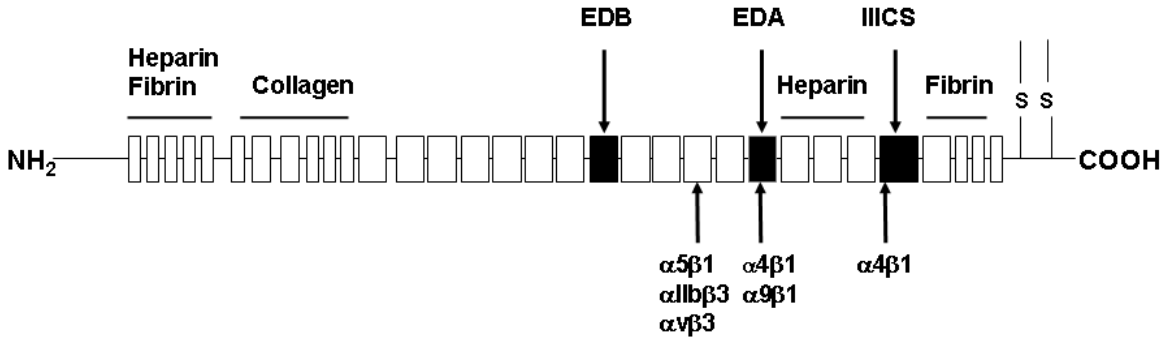

Figure 1. Murine FN schematic structure and binding sites. Various domains of FN that interact with fibrin, collagen and heparin are indicated. The alternative spliced sites EDB, EDA and IIICS domains are shown with black boxes. Integrins binding site are indicated.