**Supplemental Material**

**Roles of hypertension in the rupture of intracranial aneurysms**

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**Short title:** Hypertension and intracranial aneurysm rupture

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**Supplemental Methods**

Experiments were conducted in accordance with the guidelines approved by the University of California, San Francisco's Institutional Animal Care and Use Committee. We used 10- to 12-week-old C57BL/6J mice (Jackson Laboratory, Bar Harbor, Maine). All of the surgical procedures were performed under general anesthesia with isoflurane or a combination of ketamine and xylazine.

Intracranial aneurysms were induced by combining induced systemic hypertension and a single injection of elastase into the cerebrospinal fluid at the right basal cistern using the previously described method.[1-4]

To induce systemic hypertension, we used deoxycorticosterone acetate-salt hypertension (DOCA-salt hypertension).[2] The mice underwent unilateral nephrectomy followed one week later by the implantation of a DOCA pellet (66 mg, 28-day release); 1% sodium chloride drinking water was started on the same day as the pellet implantation.[5] The mice received a single injection of elastase (35 milli-units) into the cerebrospinal fluid at the right basal cistern on the same day as the DOCA pellet implantation.[3, 4]

Using the tail cuff method, systolic blood pressure was measured in the mice before the treatment and two and three weeks after the elastase injection.[5] Three weeks after the aneurysm induction, we euthanized the mice and perfused the animals with bromophenol blue dye. Three blinded observers assessed the formation of intracranial aneurysms by examining the Circle of Willis and its major branches under a dissecting microscope (10X). Intracranial aneurysms were operationally defined as a localized outward bulging of the vascular wall in the Circle of Willis or in its major primary branches, as previously described.[4]

Two blinded observers performed daily neurological examination using a previously described method with minor modifications.[6-9] Neurological signs were scored as followings; 0: normal function; 1: reduced eating or drinking activity demonstrated by the weight loss greater than 2 grams of body weight (approximately 10% weight loss) over 24 hours; 2: flexion of torso and forelimb upon lifting of the whole animal by the tail; 3: circling to one side but normal posture at rest; 4: leaning to one side at rest; and 5, no spontaneous activity. Mice were euthanized when the neurological score was 1-5. All asymptomatic mice were euthanized 21 days after aneurysm induction. After euthanasia, mice were perfused with phosphate-buffered saline, then with gelatin containing blue dyes to visualize the cerebral arteries. Two blinded investigators assessed brains for aneurysm formation and subarachnoid hemorrhage.

Immunohistochemistry was performed as previously described.[4, 5] The mice were dissected and perfused transcardially with saline followed by perfusion with a mixture of bromophenol blue dye and gelatin mixture. After the brain tissues were fixed 4% paraformaldehyde, the tissues were frozen in OCT compound (Tissue-Tek), and cut into 6-um sections. Sections were stained with hematoxylin and eosin (H&E). In all staining procedures we referred to positive controls. Visualization was under a microscope (BIOREVO, Keyence, California, USA). The primary antibodies we used were goat polyclonal anti-angiotensin II (Santa-Cruz), and rabbit polyclonal anti-angiotensin II type 1 receptor (Santa Cruz).

## References


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