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Translational research using a mouse model of intracranial aneurysm

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Abstract

We have developed a mouse model of intracranial aneurysm that recapitulates key features of human intracranial aneurysms. In this model, spontaneous aneurysmal rupture occurs with a predictable time course. Aneurysmal rupture in this model can be easily detected by assessing neurological symptoms. Similarly to human intracranial aneurysms, intracranial aneurysms in this model show an infiltration with inflammatory cells. This mouse model can be used to study the mechanisms and the potential preventive treatments for aneurysmal rupture.

Keywords

Intracranial aneurysms; animal model; inflammation

Introduction

Unruptured intracranial aneurysms are common in a general population. The prevalence of unruptured intracranial aneurysms in adults is estimated to be between 1 – 5%; 10–12 million Americans may be harboring unruptured intracranial aneurysms [1, 2]. More importantly, an increasing number of unruptured aneurysms are being diagnosed [3, 4].A recent report showed a 15% increase per year for the detection rate of unruptured aneurysms [5]. Such increase is believed to be due to the increased use and improved quality of brain imaging techniques such as CT (computed tomography) and MR scans (magnetic resonance imaging) [1, 6, 5].

Most unruptured aneurysms remain innocuous and asymptomatic until they rupture. However, when aneurysmal ruptured does occur, it causes subarachnoid hemorrhage, a devastating condition. A 30-day mortality after aneurysmal rupture is as high as 45% and the survivor morbidity that is close to 50% [1, 7, 8]. Therefore, the prevention of aneurysmal rupture is the goal in the management of patients with unruptured aneurysms. Surgical clipping or endovascular coiling is offered to patients with unruptured aneurysm as a prevention mean of future rupture, in case where the risk of rupture is believed to be higher than the risks associated with these procedures. These invasive procedures became significantly safer over the last two decades, but the morbidity associated with clipping or coiling of unruptured aneurysms is not still insignificant. While the outcomes undoubtedly vary among institutions, the rate of adverse outcomes including in-hospital mortality and

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discharge to a rehabilitation hospital or long-term facility is reported to be 2 – 2.5% [3, 9, 10].

Considering (1) the increasing detection rate of unruptured aneurysms, (2) the improved, but still significant adverse outcomes rate associated with coiling or clipping of unruptured aneurysm, (3) the high cost of these technically-intensive invasive therapies, and (4) limited treatment options for patients with giant aneurysm, pharmacological stabilization of aneurysms for the prevention of aneurysmal rupture may be an attractive alternative approach. The successful development of pharmacological stabilization of aneurysms is significant to our society.

While many previous studies focused on the mechanisms of the formation of intracranial aneurysms, there is the paucity of studies directly investigating mechanisms of aneurysmal rupture or pharmacological therapy of the prevention of aneurysmal rupture. This was primarily due to the lack of a suitable animal model where aneurysmal rupture spontaneously occurs with a predictable time course and at a predictable rate.

It has often been assumed that understanding the mechanisms of aneurysmal formation and growth provides insights into the mechanisms of aneurysmal rupture. This notion is based on an assumption that the processes of aneurysmal formation, growth, and rupture share the same or similar underlying mechanisms. However, there is no clear basis for such assumption. Mechanisms of aneurysmal rupture may be fundamentally different from those of formation and growth.

Recently, we have developed a mouse model of intracranial aneurysm that recapitulates key features of intracranial aneurysms, including spontaneous rupture [11, 12]. In this model, subarachnoid hemorrhage as a result of aneurysmal rupture causes neurological symptoms that can be easily detected by a simple neurological examination. Our innovative animal model allows us to directly study the mechanisms of aneurysmal rupture.

A combination of hypertension and a single injection of elastase into the cerebrospinal fluid causes intracranial aneurysm formation in mice

We have developed a mouse model of intracranial aneurysm that yields large aneurysms within a relatively short time frame [13, 12, 11]. Details of the model were presented in our published articles [13, 12, 11]. To induce intracranial aneurysm formation in mice, we combined two well-known factors associated with human intracranial aneurysms—hypertension and disruption of elastic lamina [7, 14–17]

In C57BL/6J male mice (8–10 weeks old), hypertension was induced by continuous infusion of angiotensin-II (1000 ng/kg/min) or DOCA-salt hypertension (DOCA: deoxycorticosterone acetate). Because our experience shows a lower surgical mortality with DOCA-salt hypertension compared to angiotensin-II induced hypertension, we are currently using DOCA-salt hypertension in our experiments. For DOCA-salt hypertension, mice underwent nephrectomy followed by an implantation of DOCA pellet one week later; 1% sodium chloride drinking water was started on the same day as the DOCA pellet implantation [18, 19]. Disruption of elastic lamina was induced by a single injection of elastase (35 milli-units) into the cerebrospinal fluid at the right basal cistern using a stereotaxic method on the same day as DOCA pellet implantation [11, 12]. Aneurysms were defined as a localized outward bulging of the vascular wall whose diameter is greater than 150% of the parent artery diameter [11, 12].

70–80% of the mice developed intracranial aneurysms over a four-week period [11, 12]. Large intracranial aneurysms were found mostly along the right half of the Circle of Willis and its major branches (Figure 1). Figure 1A shows an unruptured aneurysm. When we euthanized those mice that developed neurological symptoms, we found an aneurysm inside a subarachnoid hemorrhage (Figure 1B). Histologically, intracranial aneurysms in this model closely resemble human intracranial aneurysms [12, 11]. Detailed histological and immunohistochemical findings were presented in our previously published papers [11, 12].

The feasibility of using the mouse model of intracranial aneurysms to study aneurysmal rupture

To characterize the onset of neurological signs and to assess the relationship between neurological symptoms and aneurysmal rupture, we analyzed the data from 56 mice including the mice that was used in our previously publishes study [20]. After aneurysm induction, two blinded observers performed daily neurological examinations using a previously described scoring system (Grade 0: Normal function; Grade 1, Reduced eating or drinking activity demonstrated by a weight loss > 2 grams; Grade 2, Flexion of the torso and forelimbs upon lifting of the whole animal by the tail; Grade 3, Circling to one side with a normal posture at rest; Grade 4, Leaning to one side at rest; Grade 5, No spontaneous activity or death) [20–24]. The mice were euthanized when they developed neurological symptoms (score 1–5), and their brain samples were assessed for aneurysm formation and subarachnoid hemorrhage.

29 mice developed neurological signs (score 1–5) between 7 and day 16 days after aneurysm induction. When the brains of these symptomatic mice were inspected, all of them revealed intracranial aneurysms with subarachnoid hemorrhages. The mice that did not develop aneurysms were excluded from this survival curve. Four weeks after aneurysm induction, the remaining asymptomatic mice were euthanized. Among these 27 asymptomatic mice, 9 mice had unruptured aneurysms, and 18 mice had no aneurysms; none of brain samples from the asymptomatic mice showed subarachnoid hemorrhage. Therefore, the incidence of ruptured aneurysms was 52%; the incidence of unruptured aneurysms was 16%. The rupture rate (the number of mice with ruptured aneurysm divided by the number of mice with ruptured or unruptured aneurysms) was 76%. These observations indicate that a daily neurological exam using this scoring system was sensitive and specific enough to detect an aneurysmal subarachnoid hemorrhage in this model.

To verify that aneurysmal formation precedes aneurysmal rupture (i.e., unruptured aneurysms occur before aneurysmal rupture), aneurysms were induced in 8 additional mice. These mice were euthanized at day seven, a time point when aneurysmal rupture started occurring in the previous set. Of these mice, 63% of the mice had unruptured aneurysms. The overall incidence of aneurysms was similar in mice that were euthanized at day seven and mice that received follow-up at 28 days after the aneurysm induction. These observations indicate that aneurysm formation does precede aneurysmal rupture in this model. Based on these observations, we developed an experimental protocol to test pharmacological therapies for prevention of aneurysmal rupture. Six days after aneurysm induction, a pharmacological treatment for the prevention of aneurysmal rupture will begin so that the treatment affects the processes that lead to aneurysmal rupture without affecting aneurysmal formation.

Summary

We have developed a mouse model of intracranial aneurysm that recapitulates key features of human intracranial aneurysms. In this model, spontaneous aneurysmal rupture occurs

with a predictable time course. Aneurysmal rupture in this model can be easily detected by assessing neurological symptoms [20]. Similarly to human intracranial aneurysms, intracranial aneurysms in this model show an infiltration with inflammatory cells [11]. We have shown the feasibility of using this mouse model to study the mechanisms and the potential preventive treatments for aneurysmal rupture [20].

There is an increasing evidence suggesting that the modulation of inflammation may change the natural course of intracranial aneurysms and prevent aneurysmal rupture [25–27, 20]. This mouse model may provide an optimal system to conduct the preclinical test anti-inflammatory therapies for the pharmacological prevention of aneurysmal rupture.

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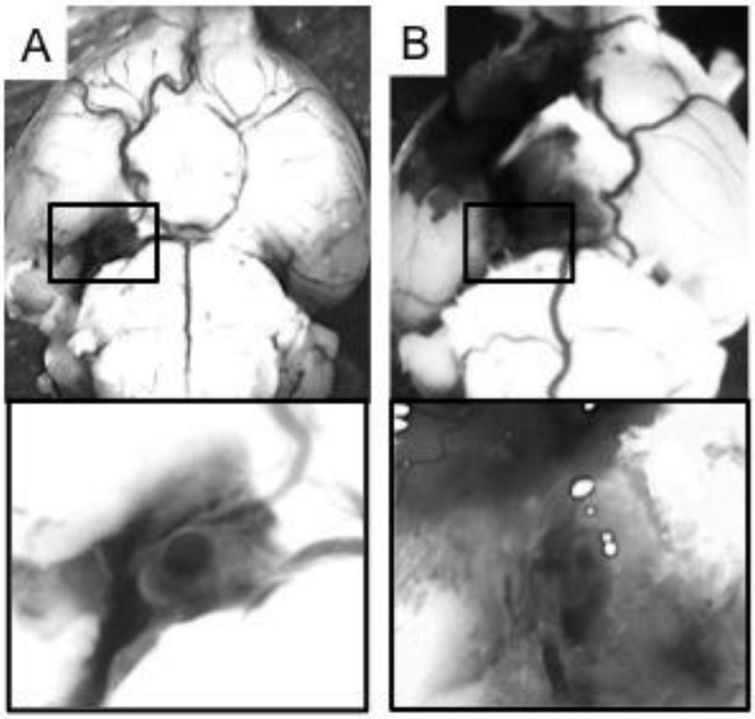

Figure 1. Representative mouse intracranial aneurysms

A: Unruptured aneurysm. B: Ruptured aneurysm. Lower panel shows the higher magnification of the upper panel.