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REVIEW

Mouse Models of Intracranial Aneurysm

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Abstract

Subarachnoid hemorrhage secondary to rupture of an intracranial aneurysm is a highly lethal medical condition. Current management strategies for unruptured intracranial aneurysms involve radiological surveillance and neurosurgical or endovascular interventions. There is no pharmacological treatment available to decrease the risk of aneurysm rupture and subsequent subarachnoid hemorrhage. There is growing interest in the pathogenesis of intracranial aneurysm focused on the development of drug therapies to decrease the incidence of aneurysm rupture. The study of rodent models of intracranial aneurysms has the potential to improve our understanding of intracranial aneurysm development and progression. This review summarizes current mouse models of intact and ruptured intracranial aneurysms and discusses the relevance of these models to human intracranial aneurysms. The article also reviews the importance of these models in investigating the molecular mechanisms involved in the disease. Finally, potential pharmaceutical targets for intracranial aneurysm suggested by previous studies are discussed. Examples of potential drug targets include matrix metalloproteinases, stromal cell-derived factor-1, tumor necrosis factor-α, the renin-angiotensin system and the β-estrogen receptor. An agreed clear, precise and reproducible definition of what constitutes an aneurysm in the models would assist in their use to better understand the pathology of intracranial aneurysm and applying findings to patients.

INTRODUCTION

Subarachnoid hemorrhage secondary to intracranial aneurysm rupture has a high mortality approaching 50% in some studies (93, 62). Although intracranial aneurysms are present in approximately 2%–5% of the population, the incidence of aneurysmal subarachnoid hemorrhage in Australia is only 8 in 100 000 patients per year (90). Intracranial aneurysm rupture is the most common cause for non-traumatic spontaneous subarachnoid hemorrhage (101). The management of unruptured intracranial aneurysms is controversial. Aneurysms deemed at low risk of rupture are typically kept under radiological surveillance, with endovascular coiling or surgical clipping offered to patients deemed to be higher risk of aneurysm rupture.

Most of the studies investigating the pathophysiology of human intracranial aneurysms have relied on specimens obtained during autopsy or surgery; however, these samples are now less commonly available due to the increasing use of endovascular treatments. Animal models of intracranial aneurysms have a potential role in improving the understanding of intracranial aneurysm development and progression, and in identifying the potential pharmaceutical therapies. Mouse models are of particular interest because of the availability of genetic modifications allowing the assessment of how different genes and pathways influence intracranial aneurysm development. This review focuses on the techniques used to prepare currently available mouse models of intracranial aneurysm, their relevance to human aneurysms and the mechanisms important in aneurysm pathogenesis in these models.

PATHOLOGY AND RISK FACTORS FOR HUMAN INTRACRANIAL ANEURYSM FORMATION

Normal human cerebral arteries have three layers: an outer collagenous adventitia, a prominent muscular media and an inner intima lined by a layer of endothelial cells. However, the tunica adventitia is extraordinarily thin in cerebral vessels and essentially disappears altogether in pial arteries with increasing depth. An internal elastic lamina separates the intima from the media. Cerebral arteries do not have external elastic lamina (82, 101) and lack vasa vasora (23, 24, 45). Instead, numerous micropores abound ("rete vasora"), which permit cerebrospinal fluid supply of glucose and waste wash-out (12, 73, 100). These micropores are dilated by vasoconstriction (49, 70, 71). Most intracranial aneurysms occur at arterial bifurcations of the circle of Willis and its major branches

where hemodynamic stress is high and turbulence of blood flow may occur (31). One main pathological feature of human intracranial aneurysms is thinning of the muscular media (37, 86). However, similar medial defects occur in at least 30% of the adult population without intracranial aneurysms (37). At the site of an intracranial aneurysm, it has been reported that the arterial wall is completely devoid of a media (82). Another main feature of human intracranial aneurysms is the destruction of the internal elastic lamina, which either is absent completely or exists as fragmentary remnants (also known as Reuterwall's tears) (82). In consequence, only the thin tunica adventitia typically remains. Infiltration of inflammatory cells has been noted within the wall of human intracranial aneurysms. Such inflammatory cells include macrophages, T lymphocytes, B lymphocytes and mast cells (20, 22, 52).

There are a number of established risk factors for intracranial aneurysm rupture such as family history, female sex, age (the sixth decade), pregnancy, labor, previous aneurysmal subarachnoid hemorrhage, excessive alcohol intake and cigarette smoking (16, 21, 42, 47, 48, 51, 55, 26, 67, 69, 76–78, 81). The morbidity and mortality associated with the surgical and endovascular treatment of unruptured intracranial aneurysm is considerable. Death or disability occurs in 7.1%–12.6% of patients within 1 year of treatment and increases exponentially with older age (97). The morbidity and mortality rate is much higher in ruptured aneurysms. For example, the International Subarachnoid Aneurysm Trial (ISAT) demonstrated that 30.6% of patients who underwent surgical treatment for ruptured aneurysms and 23.7% of patients who underwent endovascular treatment for ruptured aneurysms became dependent or died at 1 year (64). No pharmaceutical treatments are currently available to limit the main complication of intracranial aneurysm, that is, subarachnoid hemorrhage (8). Data from the international study of unruptured intracranial aneurysms suggest that patients with larger pre-existing aneurysm are at much greater risk of subarachnoid hemorrhage (97). About 12%–15% of intracranial aneurysms are familial (101). There is an increased incidence of intracranial aneurysms in patients with a family history of polycystic kidney disease, fibromuscular dysplasia, neurofibromatosis type I, tuberous sclerosis and Ehlers-Danlos syndrome type IV (19, 75, 101).

MOUSE MODELS OF INTRACRANIAL ANEURYSM

The main techniques used to induce intracranial aneurysms in mice are based on those originally employed in rats. Hashimoto *et al* established the first rat model of intracranial aneurysm in 1978 based on the hypothesis that if the hemodynamic stress imposed on a fragile cerebral arterial wall was high, intracranial aneurysms might be produced (41). Animals were treated with β-aminopropionitrile (an inhibitor of lysyl oxidase that catalyzes the cross-linking of collagen and elastin) to weaken the blood vessels and elastase was used to digest and weaken the cerebral arterial wall. Hypertension was induced by administration of deoxycorticosterone acetate and salt, or by ligation of the posterior branches of the renal arteries in combination with salt. High hemodynamic stress was induced by ligation of one common carotid artery.

Twenty-two publications that report the use of mouse models of intracranial aneurysm were identified by searching the PubMed database (1, 3–7, 9–11, 25, 43, 44, 50, 60, 65, 66, 68, 79, 85, 87, 88, 99). In these papers, five mouse models of intact intracranial aneurysm (see the following sections) and two mouse models of ruptured intracranial aneurysm (see the following sections) have been reported (Table 1). Common features of these models include induction of hypertension (eg, by renal artery ligation and angiotensin II infusion); use of techniques to weaken cerebral arteries (eg, by elastase injection) and procedures to alter cerebral blood flow (eg, by carotid artery ligation). These models are summarized in the following sections.

Ligation of a common carotid artery in combination with hypertension

A mouse model of intracranial aneurysms was first reported in 2002 by Morimoto *et al* (65). In this report, intracranial aneurysms were induced by increased cerebral hemodynamic stress and hypertension. In brief, 7- to 9-week-old C57BL/6 mice were subjected to ligation of both the left common carotid artery and the posterior branches of the right renal artery under general anesthesia. One week later, the posterior branches of the left renal artery were also ligated. One week after the second operation, 1% sodium chloride (NaCl) was substituted for drinking water (65). The surviving mice (90%) were killed 4 months later and 6 of 18 (33%) mice showed advanced stages of intracranial aneurysm evidenced by vessel wall protrusion and degradation of connective tissue. In addition, another eight mice showed early changes of intracranial aneurysm evidenced by thinning of the muscular media and discontinuity of the internal elastic lamina, but without protrusion of the vessel wall.

Ligation of a common carotid artery in combination with both hypertension and β-aminopropionitrile administration

This method was first reported by Moriwaki *et al* (66). Briefly, the left common carotid artery and the posterior branch of the left renal artery were ligated. One week later, the posterior branch of the right renal artery was ligated. Then, the mice were fed a diet containing 8% NaCl and 0.12% β-aminopropionitrile (66, 79). The experimental duration was 3–5 months. The incidence rate of intracranial aneurysm ranged from 23% to 31%.

Some variations in this model have been reported. For example, one research team reported performing only the left common carotid artery ligation in the initial operation followed by ligation of the posterior branches of both renal arteries one week later (3, 5, 6, 7, 9). Other investigations have reported using a similar model except that they ligated the main left renal artery and omitted any intervention on the right renal artery (10, 11).

The intracranial aneurysms formed by this method are small. Some authorities have defined them as micro-aneurysms, which are distinctively different from human intracranial aneurysms.

Table 1. Mouse models of intracranial aneurysms.
Abbreviation: eNOS$^{-/-}$ = endothelial nitric oxide synthase-deficient mice.

Methods	Features	References
Intact intracranial aneurysms		
Ligation of a common carotid artery in combination with hypertension	• Suitable for wild-type C57BL/6 mice • Mouse age: 7–9 weeks • Experimental duration: 4 months • 33% of mice developed intracranial aneurysms	(4, 65)
Ligation of a common carotid artery in combination with both hypertension and β-aminopropionitrile administration	• Suitable for wild-type C57BL/6 mice • Mouse age: 5–9 weeks • Experimental duration: 3–5 months • 23%–31% developed intracranial aneurysms	(3, 5–7, 9–11, 66, 79)
Ligation of a common carotid artery	• Only suitable for certain types of mice, such as eNOS$^{-/-}$ and Blotchy mice • Long experimental duration, for example, 10 months • Not suitable for wild-type C57BL/6 mice • Hypertension could increase the intracranial aneurysm incidence induced by this model	(2, 25)
Angiotensin II in combination with elastase	• Suitable for wild-type C57BL/6 mice • Short experimental duration, for example, 2–3 weeks • High incidence rate: up to 77% • Incidence was elastase concentration-dependent	(50, 68)
Malaria infection	• Intracranial aneurysms were induced by infection with malaria parasites • This method was not well established and not commonly used by researchers to investigate intracranial aneurysms	(99)
Ruptured intracranial aneurysms		
Deoxycorticosterone acetate-salt in combination with elastase	• Suitable for wild-type C57BL/6 mice • Mouse age: 8–10 weeks • Short experimental duration: 4 weeks • High rupture rate: 50%–63% • High intracranial aneurysm rate: 62%–82%	(60, 85, 87, 88)
Ligation of the left common carotid artery and the right renal artery in combination with angiotensin II, β-aminopropionitrile, and elastase	• Suitable for wild-type C57BL/6 mice • Mouse age: 7–10 weeks • Short experimental duration: 3 weeks • High rupture rate: up to 60% • High intracranial aneurysm rate: 89%–100%	(43, 44)

Ligation of a common carotid artery

Intracranial aneurysms can be induced by ligating a common carotid artery in certain strains of mice. For example, two of six (33%) endothelial nitric oxide synthase-deficient (eNOS$^{-/-}$) mice developed intracranial aneurysms 13 months after ligation of the left common carotid artery; however, none of the 30 wild-type C57BL/6 mice did (2). Hypertension in eNOS$^{-/-}$ mice may predispose these mice to develop intracranial aneurysms (95).

Blotchy mice have an inherited disorder of connective tissue synthesis (74). Ligation of the left carotid artery for 10 months induced dilation of cerebral arteries in 17% of Blotchy mice (25). Hypertension was reported to increase the incidence of intracranial aneurysm in this model. For example, concurrent common carotid artery ligation and hypertension induction was shown to result in the development of intracranial aneurysms in 38% of Blotchy mice (25).

Angiotensin II in combination with elastase

This model was reported in 2009 by Nuki *et al* (68). Briefly, 8- to 10-week-old C57BL/6 mice were anesthetized and then an osmotic pump, which delivered angiotensin II at 1000 ng/kg/min was implanted under the dorsal skin to induce hypertension. Mice were held in place using a stereotactic frame and 35 milliunits of elastase was injected into the right basal cistern (68). Seventy-seven percent of the mice developed intracranial aneurysms within 2 weeks. The same study also reported that the incidence of intracranial aneurysm was dependent on the elastase dose. The incidence rates of intracranial aneurysms were 10% and 30% when 3.5 and 17 milliunits of elastase, respectively, were delivered (68). Intracranial aneurysms were characterized by media loss, elastic lamina degeneration and inflammatory cell infiltration (68). Another study reported that employing 17 milliunits of elastase together with 1000 ng/kg/min angiotensin II could induce intracranial aneurysm formation in 60% of animals within 3 weeks (50).

Malaria infection

CF1 and A/J mice infected with the mouse malaria strain *Plasmodium berghei yoelii 17x* showed intravascular sequestration of parasite-infected erythrocytes and blockage of brain capillaries, which led to intracranial aneurysm formation (99). This mouse model may mimic the rare clinical situation that infection can lead to intracranial aneurysm formation. However, this mouse model is not well established and is not commonly used by researchers to investigate intracranial aneurysms.

Deoxycorticosterone acetate-salt in combination with elastase

This intracranial aneurysm rupture model was induced in C57BL/6 mice. In brief, 8- to 12-week-old mice underwent unilateral nephrectomy followed by the implantation of a deoxycorticosterone acetate pellet 1 week later. Mice received 1% NaCl drinking water on the same day as the deoxycorticosterone acetate pellet implantation. Mice received a single injection of elastase (25–35 milliunits) to the right basal cistern on the same day as the deoxycorticosterone acetate pellet implantation (60, 87, 88). Further, 50%–63% of mice developed ruptured intracranial aneurysms between day 7 and day 11 after aneurysm induction, and the overall incidence of intracranial aneurysm was 62%–82% within 28 days after induction commenced (60, 85, 88).

A key advantage of intracranial aneurysm rupture models is that they can be used to test mechanisms involved in and potential treatments for the prevention of aneurysm rupture (60). The prevention of aneurysmal rupture is clinically important as intracranial aneurysm rupture is associated with significant morbidity and a high mortality (14, 97).

Ligation of the left common carotid artery and the right renal artery in combination with angiotensin II, β-aminopropionitrile and elastase

This model was reported by Hosaka *et al* (44). The left common carotid artery and the right renal artery of 7- to 10-week-old C57BL/6 mice were ligated. One week later, the mice were fixed in a stereotactic frame and elastase (10, 50, 100 or 200 milliunits) was injected into the right basal cistern. After the elastase injection, a micro-osmotic pump that delivered 1000 ng/kg/min angiotensin II was implanted subcutaneously. The mice were fed a diet containing 8% NaCl and 0.12% β-aminopropionitrile (44). Moreover, 90% of the mice receiving 10 milliunits of elastase developed intracranial aneurysms, whereas the incidence was 100% in the mice receiving higher doses of elastase. The rate of intracranial aneurysm rupture within 3 weeks was 20, 40, 60 and 50% in mice that receiving 10, 50, 100, or 200 milliunits of elastase, respectively (44). In another study from the same group using the same methods, it was reported that when 200 milliunits elastase was used, intracranial aneurysms were formed in 89% of mice and the rupture rate was 33% within 3 weeks (43).

Other mice models of intracranial aneurysm have been proposed, which focused on the development of saccular aneurysms outside the skull. It has been reported that saccular aneurysms can be induced by transplanting syngeneic thoracic aorta end-to-side to the abdominal aorta in mice (34). This extracranial saccular aneurysm model may be useful to test endovascular treatments for intracranial aneurysm. Mice have very small intracranial arteries, meaning that the other models of intracranial aneurysm described earlier are unlikely to be able to be used for endovascular therapy testing.

DEFINITIONS OF INTRACRANIAL ANEURYSM USED IN PREVIOUS MOUSE MODEL STUDIES

The methods used to define intracranial aneurysms in previous studies have varied. Ten of the studies used macroscopic assessment of the Circle of Willis and defined intracranial aneurysms as a localized outward bulging of the vascular wall (2, 25, 43, 44, 50, 60, 68, 85, 87, 88). Among these studies, Starke *et al* defined an intracranial aneurysm as a localized outward bulging of the vascular wall of which the diameter was >150% of the diameter of the parent artery (85). Nuki *et al* defined an intracranial aneurysm as a localized outward bulging of the vascular wall, of which the diameter was >150% of the diameter of the basilar artery (68). In the intracranial aneurysm rupture models, the aneurysms were simply categorized as non-ruptured and ruptured (43, 44, 60, 85, 87, 88). To assist the macroscopic assessment of intracranial aneurysms, the brain was perfused with a bromophenol blue-containing gelatin in some studies (44, 60, 87, 88).

Another 10 studies used light microscopy of arterial sections to define intracranial aneurysms as an outward bulging of the arterial wall with fragmentation or disappearance of the internal elastic lamina (3–7, 9, 10, 65, 66, 79). In these studies, histological assessments were performed with a variety of techniques including orcein or Elastica van Gieson staining methods.

The histological analyses reported in these studies were often conducted by independent researchers who were blinded to the experimental protocol in order to minimize the bias (2, 3, 6, 46, 68, 85). The reproducibility of the assessments was not reported in most studies. Nuki *et al* reported that the angiotensin II and elastase model was reproducible, as two independent batches of experiments showed similar intracranial aneurysm induction rates (77% vs. 70%) (68). None of the reports however provided information on intra or inter-observer reproducibility in defining an intracranial aneurysm. There was limited information provided on how macroscopic bulges equivalent to an intracranial aneurysm were defined and when diameters were required for a definition the method of measuring this was often absent in the report.

Early aneurysmal changes have also been described in some of the included studies through analysis of sections of intracranial arteries stained for elastin as discontinuity of the internal elastic lamina without apparent outward bulging of the arterial wall (3, 6, 9, 10, 65, 66, 79). Whether these early aneurysmal changes progress to true aneurysm formation is not yet known. To prepare the tissue for histological staining of elastin, the mice were commonly perfusion fixed with paraformaldehyde or glutaraldehyde and the brain was kept in the fixative overnight before being embedded in resin (2, 65). In some reports, the tissue was embedded in optimal cutting temperature compound (OCT) and sections from frozen tissue were used to stain elastin (3, 9, 43, 66). An agreed clear, precise and reproducible definition of what

constitutes an aneurysm in the models would assist in their use to better understand the pathology of intracranial aneurysm and apply findings to patients.

THE MOLECULAR MECHANISMS INVOLVED IN INTRACRANIAL ANEURYSM DEVELOPMENT IN THESE MOUSE MODELS

Mouse models are potentially useful tools for investigating the molecular mechanisms involved in intracranial aneurysm pathogenesis. Mechanisms highlighted include extracellular matrix degradation and inflammation and key molecules implicated in intracranial aneurysm formation are described below.

Matrix metalloproteinases (MMPs)

One of the main pathological features of intracranial aneurysms is the disappearance or discontinuity of the internal elastic lamina (86). The structural integrity of the vessel wall depends on a balance between synthesis and degradation of the extracellular matrix. MMPs can degrade most of the arterial extracellular matrix components including elastin and collagen. In human intracranial aneurysms, the expression of MMPs (eg, MMP-2 and MMP-9) has been reported to be increased (17, 18, 54), suggesting that increased MMPs may contribute to the extracellular matrix disruption associated with intracranial aneurysms. Deficiency in MMP-9, but not MMP-2, reduced the incidence of intracranial aneurysm in one mouse model induced by angiotensin II in combination with elastase, suggesting that MMP-9, but not MMP-2, is important in the development of intracranial aneurysm in mice (68).

Tissue inhibitors of matrix metalloproteinases (TIMPs)

TIMPs are considered the key inhibitors of MMPs in tissue (15). Abundant TIMP expression has been detected in human intracranial aneurysms (54). TIMP-1 or TIMP-2 deficiency promoted aneurysm development and progression within a mouse model induced by ligation of a common carotid artery in combination with both hypertension and β-aminopropionitrile administration (3). Deficiency of TIMP-1 or TIMP-2 did not affect blood pressure nor macrophage infiltration. MMP-9 activity in TIMP-$1^{-/-}$ mice and MMP-2 activity in TIMP-$2^{-/-}$ mice were elevated compared with wild-type mice (3), suggesting that the preventive effects of TIMP-1 and TIMP-2 on intracranial aneurysm were derived from their ability to inhibit the activity of MMPs.

Inducible nitric oxide synthase (iNOS)

iNOS is induced during inflammation. It can produce large quantities of nitric oxide upon stimulation, which can further react with superoxide to form peroxynitrite (a highly reactive oxidant) to promote inflammation (38). iNOS expression has been reported to be upregulated in human intracranial aneurysm (36). iNOS was reported to be induced within the media and adventitia of the aneurysmal wall in mice (79). iNOS deficiency did not change the incidence of intracranial aneurysm in a mouse model induced by ligation of a common carotid artery in combination with both hypertension and β-aminopropionitrile administration. However, the size of intracranial aneurysms was smaller in iNOS-deficient mice compared with wild-type mice (12.7 ± 2.1 μm vs. 32.7 ± 4.6 μm, $P < 0.05$) (79), associated with a decrease in vascular smooth muscle cell (VSMC) apoptosis. This finding suggests that iNOS may promote intracranial aneurysm progression.

Interleukin-1β (IL-1β)

IL-1β is a pro-inflammatory cytokine highly produced by mononuclear phagocytes in response to injury and infection and is regarded as a master regulator of neuroinflammation (13). One study reported that in patients with aneurysmal subarachnoid hemorrhage, subjects carrying the T/T genotype of the *IL-1β* gene showed a significant increase in the Hunt and Hess scores at hospital admission and a significant reduction in 6-month Glasgow Outcome Scale scores (30), suggesting that IL-1β may modify the complications of intracranial aneurysms. IL-1β mRNA levels in cerebral arteries were increased in wild-type C57BL/6 mice at both 2 weeks and 3 months after induction of intracranial aneurysm by ligation of a common carotid artery in combination with both hypertension and β-aminopropionitrile administration (66). The incidence of advanced aneurysm was significantly lower in IL-$1\beta^{-/-}$ compared with wild-type mice (66), associated with a reduction in apoptosis as indicated by a decrease in single-stranded DNA staining as well as terminal deoxynucleotidyl transferase dUTP nick end labeling positive cells. This study suggests that IL-1β may promote intracranial aneurysm formation.

Nuclear factor-kappa B (NF-κB)

NF-κB is a key transcriptional factor regulating the expression of a variety of genes including vascular cell adhesion molecule-1 (VCAM-1), monocyte chemoattractant protein-1 (MCP-1), iNOS and MMPs (72). NF-κB was reported to be activated in the arterial wall of human intracranial aneurysms (4). The incidence of intracranial aneurysm in NF-κB-deficient mice was lower compared with wild-type mice (0% vs. 30%) 5 months after aneurysm induction (4). The mRNA levels of MCP-1, VCAM-1, MMP-2, MMP-9, IL-1β and iNOS were elevated within aneurysms in wild-type mice, whereas the mRNA levels of these molecules were not upregulated in NF-κB-deficient mice. In addition, macrophage infiltration was much less in NF-κB-deficient mice compared with wild-type mice (4).

MCP-1

MCP-1 plays a critical role in monocyte/macrophage recruitment in various vascular diseases such as atherosclerosis (29). In human intracranial aneurysms, MCP-1 was reported to be expressed in endothelial cells, VSMCs and macrophages (6). MCP-1 expression was reported to be upregulated in the wall of early stage intracranial aneurysms in mice (6). MCP-1-deficient mice exhibit a significant decrease in intracranial aneurysm formation (6, 50), associated with a decrease in both macrophage accumulation and the expression of genes implicated in aneurysm formation including MMP-9, MMP-2 and iNOS (6).

NADPH oxidase

Superoxide is a major mediator of various inflammatory cascades and is implicated in the pathogenesis of various diseases including atherosclerosis. Superoxide is produced by enzymatic oxidation. One of the enzymes responsible for superoxide production is NADPH oxidase. In NADPH oxidase-deficient mice, the intracranial aneurysm size was smaller compared with that in wild-type mice, which was associated with a decrease in oxidative stress, macrophage infiltration, and a downregulation of NF-κB and MCP-1 (7).

Prostaglandin E2 (PGE2) receptors

PGE2 is a prostanoid with a pro-inflammatory effect. Cyclooxygenase-2 (COX-2) and PGE synthase-1 (PGES-1) are responsible for PGE2 production (91). PGE2 exerts its actions through a family of G protein-coupled receptors, that is, EP1, 2, 3 and 4 receptors. In human intracranial aneurysms, the expressions of COX-2 and PGES-1 have been reported to be increased (10, 40). In addition, PGE receptor EP2 expression has been identified within endothelial cells of human intracranial aneurysms (10).

PGE2 receptor-deficient mice showed a decrease in intracranial aneurysm formation, which was associated with a decrease in macrophage infiltration, reduced NF-κB activation, and a decrease in the expression of MCP-1, IL-1β and MMP-2 (10).

Tumor necrosis factor-α (TNF-α)

TNF-α signaling has been implicated in the pathogenesis of intracranial aneurysm in humans. For example, the plasma TNF-α concentration was higher in patients with intracranial aneurysms compared with that in age-matched healthy controls (102). Single nucleotide polymorphisms in the TNF-α gene have been reported to be associated with the presence of intracranial aneurysms in a Japanese population (58). TNF-α deficient mice showed a decrease in intracranial aneurysm formation and rupture (85). In addition, TNF-α receptor superfamily member 1α-deficient mice showed a decrease in intracranial aneurysm formation, which was associated with a decrease in NF-κB activation, MCP-1 and COX-2 expression, and macrophage infiltration (11).

eNOS

eNOS catalyzes L-arginine to form nitric oxide which can dilate arteries and reduce the hemodynamic stress imposed on arterial walls. Some human studies have reported that eNOS genetic polymorphisms are associated with intracranial aneurysm rupture (53). eNOS seems to protect mice against intracranial aneurysm formation. In a model of intracranial aneurysms induced by ligation of the left common carotid artery, eNOS$^{-/-}$ mice were more prone to intracranial aneurysm development (2). Histological analysis showed that aneurysms were characterized by internal elastic lamina destruction, subendothelial collagen deposition and inflammatory cell infiltration (2).

Another study however reported that the incidence of intracranial aneurysm in eNOS$^{-/-}$ mice was not different from wild-type mice. This study used a different model in which aneurysms were induced by carotid artery ligation in combination with hypertension and β-aminopropionitrile administration. Interestingly, the neuronal NOS (nNOS) expression was increased in the intracranial aneurysmal wall in the eNOS$^{-/-}$ mice (9), suggesting that nNOS may compensate for the deficiency in eNOS.

Apolipoprotein E (ApoE)

ApoE plays an important role in cholesterol metabolism by serving as a cholesterol transporter and promoting cholesterol efflux and degradation (59, 61). ApoE can also regulate inflammatory and oxidative processes (94, 98). Therefore, ApoE might be expected to be involved in intracranial aneurysm formation. However, one study showed that ApoE had no effect on the intracranial aneurysm formation in mice (5). Further, 16% of C57BL/6 wild-type mice developed advanced intracranial aneurysms compared to 17% of ApoE$^{-/-}$ mice. Similarly, 32% of wild-type mice developed early aneurysmal changes compared to 28% of ApoE$^{-/-}$ mice (5). This was in agreement with a clinical report which showed that ApoE alleles were not associated with the incidence of intracranial aneurysm (63). These results suggest that ApoE does not play a major role in intracranial aneurysm formation.

SIMILARITY BETWEEN MOUSE MODEL AND HUMAN INTRACRANIAL ANEURYSMS

Mouse models of intracranial aneurysms mimic some key features of the disease in humans. They share similarities in the disease location and pathology, including degradation of the internal elastic lamina, thinning of the media, inflammation and molecular changes.

Aneurysm location

Human intracranial aneurysms typically occur at the bifurcations of major vessels around the circle of Willis and its major branches (83, 96). The common sites of human intracranial aneurysms include the internal carotid artery, the anterior communicating artery, the middle cerebral artery, the posterior communicating artery, the posterior cerebral artery, the basilar artery and the vertebral artery (101). The majority of the intracranial aneurysms in mice models are also located at bifurcations along the circle of Willis and its major branches, such as at the anterior cerebral artery-anterior communicating artery bifurcation, the anterior cerebral artery-olfactory artery bifurcation, the posterior cerebral artery or the basilar artery (5, 7, 9, 10, 65, 66, 68, 79), which are generally in accordance with the locations of human intracranial aneurysms.

Degradation of the internal elastic lamina

Like human cerebral arteries, mouse cerebral arteries lack an external elastic lamina (84). Human intracranial aneurysms are characterized by destruction of the internal elastic lamina, which is either absent completely or exists as fragmentary remnants (1, 80, 82). Degradation of the internal elastic lamina is reported within mouse models of intracranial aneurysms (2, 10, 25, 44, 65, 68).

Thinning of the tunica media

The wall of an unruptured human intracranial aneurysm is characterized by myointimal hyperplasia (35). The wall of a ruptured human intracranial aneurysm is often devoid of smooth muscle cells (32, 33, 35, 52, 82, 92). The loss of VSMCs may result from increased apoptosis within the aneurysmal wall (39, 89). Mouse intracranial aneurysms show thinning of the tunica media and VSMC apoptosis (65, 66, 79) similar to human aneurysms.

Inflammation

Human intracranial aneurysms show evidence of infiltration by macrophages, T-cells, B-cells and mast cells (20, 22, 28, 33, 52, 92). In addition, there is upregulation of a range of molecules associated with inflammation. For example, complement (eg, C3c, C9), immunoglobulin (eg, IgG, IgM) and VCAM-1 were reported to be upregulated in human intracranial aneurysms (22, 52). Mouse models of intracranial aneurysm also show increased inflammation within the aneurysmal wall (2–4, 6, 7, 9, 44, 50, 68), and deficiencies in some pro-inflammatory genes (eg, NF-κB and MCP-1) can inhibit intracranial aneurysm formation.

Molecular mechanisms

Using mouse models of intracranial aneurysms, a number of molecules have been implicated in intracranial aneurysm formation. These molecules are mainly involved in extracellular matrix degradation and inflammation. These molecules may also be involved in human intracranial aneurysm pathogenesis (see the section The molecular mechanisms involved in intracranial aneurysm development in these mouse models).

POTENTIAL PHARMACEUTICAL THERAPIES SUGGESTED BY RESEARCH ON MOUSE INTRACRANIAL ANEURYSMS

Mouse models of intracranial aneurysm have identified some potential pharmaceutical targets (Table 2).

MMP inhibition

Doxycycline, a broad-spectrum MMP inhibitor, reduced the incidence of intracranial aneurysms from 70% to 10% in a mouse model of intracranial aneurysm (68). In addition, doxycycline and minocycline inhibited the rupture of intracranial aneurysms induced by deoxycorticosterone acetate and elastase (60). However, the same study showed that SB-3CT, a potent and selective inhibitor of MMP-2 and MMP-9, did not inhibit the rupture of intracranial aneurysms, although the reason for this was not identified.

Table 2. Potential therapies suggested by research on mouse intracranial aneurysms.
Abbreviations: MMP = matrix metalloproteinase; RAS = renin-angiotensin system; SDF-1 = stromal cell-derived factor-1; TNF-α = tumor necrosis factor-α.

Therapies	Findings	Animal models used	References
MMP inhibition	Doxycycline, a broad-spectrum MMP inhibitor, reduced the incidence of intracranial aneurysm in mice.	Angiotensin II in combination with elastase	(60, 68)
	Doxycycline and minocycline inhibited the rupture of intracranial aneurysms induced by deoxycorticosterone acetate-salt and elastase in mice.	Deoxycorticosterone acetate-salt in combination with elastase	
SDF-1 inhibition	Inhibition of SDF-1 by intravenous administration of SDF-1 antibody significantly reduced intracranial aneurysm formation from 89% to 33%, which is likely due to a decrease in angiogenesis and inflammation.	Ligation of the left common carotid artery and the right renal artery in combination with angiotensin II, β-aminopropionitrile and elastase	(43)
TNF-α inhibition	Pre-treatment with 3,6′-dithiothalidomide, a synthesized TNF-α inhibitor, decreased intracranial aneurysm formation and rupture in mice. In addition, treatment with 3,6′-dithiothalidomide after intracranial aneurysm formation decreased rupture of pre-formed aneurysms.	Deoxycorticosterone acetate-salt in combination with elastase	(85)
RAS inhibition	Inhibition of angiotensin-converting enzyme with captopril or inhibition of the angiotensin type I receptor with losartan decreased the incidence of ruptured aneurysms without affecting blood pressure.	Deoxycorticosterone acetate-salt in combination with elastase	(87)
Blood pressure-lowering agents	Treatment with hydralazine normalized blood pressure of mice with intracranial aneurysms induced by deoxycorticosterone acetate-salt and elastase. This treatment significantly reduced the incidence of ruptured intracranial aneurysms.	Deoxycorticosterone acetate-salt in combination with elastase	(87)
Estrogen receptor-β agonists	Treatment with diarylpropionitrile, an estrogen receptor-β agonist, protected mice against aneurysm rupture. The protective estrogen receptor-β stimulation was mediated by nitric oxide production.	Deoxycorticosterone acetate-salt in combination with elastase	(88)

Stromal cell-derived factor-1 (SDF-1) inhibition

SDF-1 is a chemokine that promotes angiogenesis and inflammation (56, 57). Human intracranial aneurysms show increased SDF-1 expression (43). Inhibition of SDF-1 by intravenous administration of an SDF-1 antibody significantly reduced intracranial aneurysm formation from 89% to 33% (43), which is likely due to decreased angiogenesis and inflammation.

TNF-α inhibition

Administration of 3,6′-dithiothalidomide, a synthesized TNF-α inhibitor, decreased intracranial aneurysm formation and rupture in mice (85). In addition, administration of 3,6′-dithiothalidomide reduced intracranial aneurysm rupture in a mouse model (85).

Renin-angiotensin system inhibition

The expression of angiotensin II and the angiotensin II type 1 receptor has been reported to be increased within mouse intracranial aneurysms (87). Inhibition of angiotensin converting enzyme with captopril or inhibition of the angiotensin type I receptor with losartan decreased the incidence of ruptured aneurysms without affecting blood pressure (87). These results suggest that inhibiting the renin-angiotensin system may be a potential therapy to limit intracranial aneurysm rupture.

Blood pressure lowering agents

Administration of hydralazine (50 mg/day per day in the drinking water) normalized blood pressure of mice with intracranial aneurysms induced by deoxycorticosterone acetate and elastase (87). This intervention did not affect the formation of intracranial aneurysms; however, it significantly reduced the incidence of ruptured aneurysms (87). In addition, discontinuation of deoxycorticosterone six days after the induction of intracranial aneurysms lowered blood pressure, which was accompanied with a decrease in the incidence of aneurysm rupture. These results suggest that lowering blood pressure may be a useful therapeutic strategy to prevent intracranial aneurysm rupture.

Estrogen receptor-β agonists

Clinical studies suggest that postmenopausal women have a higher incidence of intracranial aneurysm rupture than premenopausal women (27). α and β estrogen receptors are reported to be expressed in human intracranial aneurysms (88). These results suggest that estrogen signaling may be involved in intracranial aneurysm rupture. Ovariectomized female mice with pre-formed intracranial aneurysms that received estrogen had reduced incidence of aneurysm rupture. The protective effect of estrogen administration was abolished by administration of a nonselective estrogen receptor antagonist (88). Stimulation of estrogen receptor-β with diarylpropionitrile, but not of the estrogen receptor-α with propyl pyrazole triol, protected mice against intracranial aneurysm rupture (88). The protective effect of estrogen receptor-β stimulation was mediated by nitric oxide production as evidenced by the fact that the protective effect of diarylpropionitrile disappeared after co-administration of N^{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME) to inhibit nitric oxide formation (88).

SUMMARY

Five mouse models of intact and two mouse models of ruptured intracranial aneurysms have been previously reported. These mouse models share similarities with human intracranial aneurysms in terms of aneurysm location and pathology. These animal models are potentially useful tools for investigating the pathogenesis of intracranial aneurysm. Research employing these models has identified some potential pharmaceutical therapies for human intracranial aneurysm, including inhibition of MMPs, SDF-1, TNF-α and the renin-angiotensin system. The potential value of blood pressure lowering and administration of estrogen receptor-β agonists in limiting complications of intracranial aneurysms has also been suggested. It however remains to be seen how this mouse model data will translate to humans.

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CONFLICT OF INTEREST

The authors have no conflicts of interest or financial ties to disclose.

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