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Intracranial Aneurysm as a Macrophage-mediated Inflammatory Disease

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Abstract

Subarachnoid hemorrhage (SAH) is mainly attributable to the rupture of intracranial aneurysms (IAs). Although the outcome of SAH is considerably poor in spite of the recent intensive medical care, mechanisms regulating the progression of IAs or triggering rupture remain to be clarified, making the development of effective preemptive medicine to prevent SAH difficult. However, a series of recent studies have been expanding our understanding of the pathogenesis of IAs. These studies have suggested the crucial role of macrophage-mediated chronic inflammation in the pathogenesis of IAs. In histopathological analyses of IA lesions in humans and induced in animal models, the number of macrophages infiltrating in lesions is positively correlated with enlargement or rupture of IAs. In animal models, a genetic deletion or an inhibition of monocyte chemotactic protein-1, a major chemoattractant for macrophages, or a pharmacological depletion of macrophages consistently suppresses the development and progression of IAs. Furthermore, a macrophage-specific deletion of *Ptger2* (gene for prostaglandin E receptor subtype 2) or a macrophage-specific expression of a mutated form of IκBα which inhibits nuclear translocation of nuclear factor κB significantly suppress the development of IAs, supporting the role of macrophages and the inflammatory signaling functioning there in the pathogenesis of IAs. The development of drug therapies suppressing macrophage-mediated inflammatory responses *in situ* can thus be a potential strategy in the pre-emptive medicine targeting SAH. In this manuscript, we summarize the experimental evidences about the pathogenesis of IAs focused on inflammatory responses and propose the definition of IAs as a macrophage-mediated inflammatory disease.

Key words: intracranial aneurysm, macrophage, chronic inflammation, EP2, NF-kB

Introduction

Stroke is one of the leading causes of death in developed countries, and subarachnoid hemorrhage (SAH) accounts for about 10% of stroke death in Japan. The mortality rate of SAH is as high as 50% in spite of the intensive medical care.[1)] The onset of SAH is mainly due to rupture of pre-existing intracranial aneurysms (IAs).[1)] As a large number of IAs are incidentally found by MRA examination[2)] especially in Japan, a pre-emptive medical care can be applied for these incidentally found IAs to prevent SAH resulting in lowering the morbidity and mortality. The treatment modalities for IAs to date, however, are limited to surgical interventions (i.e. micro-neurosurgical clipping or endovascular coiling). As surgical interventions include a risk of complications in nature, an indication for these invasive treatments has been carefully determined on a case-to-case basis considering an estimated rate of rupture in each case by characteristics of patients (e.g. age or comorbidity), those of IAs (e.g. size, shape, or location), and life expectancy of each patient.[2–5)] However, unfortunately, treatment interventions are sometimes insufficient and inappropriate because our estimation of rupture risk is not yet satisfactory. Such a situation is partially because of the fact that detailed mechanisms underlying the pathogenesis of IAs remain to be elucidated. The development of a novel medical therapy is mandatory for social health.

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IAs as a Macrophage-mediated Chronic Inflammatory Disease Affecting Intracranial Arteries

The histopathological studies investigating surgically dissected human IA specimens have defined IAs as an out-bulging lesion affecting intracranial arteries with the disruption of the internal elastic lamina and thinning of the media.[6,7] Furthermore, the presence of inflammatory cells (e.g. macrophages) in IA walls has also been demonstrated, indicating the involvement of inflammatory responses in the pathogenesis of the disease.[7–9] However, the precise contribution of inflammatory responses to the pathogenesis remains to be elucidated only from human studies. The development of animal models which well mimics the process of the disease development in humans[10] has provided experimental evidences supporting the contribution of inflammatory responses.

Histopathological analyses of IA lesions induced in rodent models[10,11] have also demonstrated the presence of inflammatory cells especially CD68-positive macrophages as in human IAs,[12] indicating the role of macrophages in the formation and progression of IAs. Such a notion is further supported by the following studies. A genetic deletion or an inhibition of monocyte chemotactic protein-1 (MCP-1), a major chemoattractant protein for macrophages, or a pharmacological depletion of macrophages by Clodronate liposome remarkably suppresses the development and enlargement of IAs,[13,14] suggesting the crucial role of macrophage-mediated inflammatory responses in the pathogenesis of IAs. Because intracranial arteries lack vasa vasorum in the adventitia, macrophages and other inflammatory cells seem to infiltrate into intracranial arterial walls across endothelial cells. For the *trans*-endothelial migration of macrophages, the disruption of inter-cellular junctions between endothelial cells, a physiological and structural barrier for macrophage infiltration, should be essential. As the activation of Sphigosine-1-phosphate receptor type 1 ($S1P_1$) signaling strengthens the barrier between endothelial cells and simultaneously reduces the number of macrophages infiltrating in IA lesions,[15] *trans*-endothelial migration may be a major machinery to accumulate macrophages in lesions. Furthermore, the activation of $S1P_1$ suppresses the enlargement of IAs, further suggesting the crucial role of infiltrating macrophages across endothelial barrier in the progression of IAs.[15]

As a genetic deletion of nuclear factor κB (NF-κB) p50 subunit or a pharmacological inhibition of NF-κB significantly suppresses expressions of pro-inflammatory genes in lesions and development and enlargement of IAs,[16–18] transcription factor NF-κB and its downstream factors play a crucial role in the pathogenesis of IAs. Pro-inflammatory factors induced by the activation of NF-κB in lesions and contributing to the disease include IL-1β,[19] cyclooxygenase-2 (COX-2),[20] inducible nitric oxide synthase[21,22] and matrix metalloproteinase-9.[12] Furthermore, prostaglandin E (PGE) receptor subtype 2 (EP2) is identified as the up-stream factor activating NF-κB in lesions, because EP2 signaling can activate NF-κB in *in vitro* and the activation of NF-κB in lesions can be suppressed in a genetic deletion of this receptor in mice.[20] Because a macrophage-specific deletion of *Ptger2* (which encodes EP2) or a macrophage-specific expression of a mutated form of IκBα which inhibits a nuclear-translocation of NF-κB significantly suppresses the development of IAs in mice, prostaglandin E_2–EP2–NF-κB signaling in macrophages plays the crucial role in the process of IA development.[23] Here, intriguingly, a deletion of EP2 specifically in macrophages can almost completely suppress a macrophage infiltration and the activation of NF-κB in whole lesions, suggesting the role of EP2 signaling in macrophages in the maintenance of inflammatory responses in lesions. In *in vitro* experiments, although EP2 signaling alone can activate NF-κB and thus evoke NF-κB-mediated inflammation, the ability to induce pro-inflammatory factors is much weaker than that of other pro-inflammatory cytokines like TNF-α.[23] Instead, EP2 signaling co-operates with TNF-α and amplifies expressions of pro-inflammatory genes induced by TNF-α.[23] As COX-2 is included in genes whose expression is amplified by EP2 signaling, the positive feedback loop containing COX-2–PGE_2–EP2 signaling cascade is thus formed, making inflammation once triggered being amplified and prolonged.[23] Furthermore, EP2 signaling stabilizes *CCL2* mRNA, which encodes MCP-1, through activating RNA-binding protein HuR and then enhances MCP-1 production.[23] Therefore, EP2 signaling functioning in macrophages forms a self-amplification loop among macrophages and contributes to the preparation of inflammatory microenvironment leading to IA formation and progression.[23] Clinical relevance of above results is demonstrated in immunohistological analyses of human IAs, in which expressions of COX-2 and EP2 are positively correlated with the number of macrophages infiltrated in lesions.[20,23]

A series of animal studies and studies using human specimens has demonstrated the crucial role of macrophage-mediated chronic inflammation in the pathogenesis of IAs. The development of drug therapies suppressing macrophage-mediated inflammatory responses thus becomes realistic.[11]

Drug Therapy for IAs Targeting Chronic Inflammation

A series of studies have successfully defined IAs as a macrophage-mediated chronic inflammatory disease. Drugs targeting macrophages or factors mediating inflammatory responses in lesions are thus reasonable candidates for the treatment of IAs. In fact, recent studies have demonstrated the inhibitory effect of anti-inflammatory drugs on the development or progression of IAs in animal models (Table 1).[12,15–18,20,21,23–48]

Case-control studies recently published have demonstrated that the usage of statins (HMG-CoA reductase

Table 1 Potential therapeutic targets fot the treatment of intracranial aneurysms demonstrated in rodent models

Therapeutic target	Drug	Animal	Effect on aneurysmal pathogenesis			Author (year)
			Formation	Enlargement	Rupture	
HMG-CoA reductase	Simvastatin	Sprague–Dawley rat		↓		Aoki et al. (2008)[17]
	Pitavastatin	Sprague–Dawley rat		↓		Aoki et al. (2009)[18]
	Pravastatin	Sprague–Dawley rat		↓		Kimura et al. (2010)[34]
NF-κB	NF-κB decoy oligodeoxynucleotide	Sprague–Dawley rat	↓	↓		Aoki et al. (2007)[16]
	Nifedipine	Sprague–Dawley rat		↓		Aoki et al. (2008)[24]
Cyclooxygenese (COX)	Aspirin	Sprague–Dawley rat		↓		Li et al. (2015)[35]
	Aspirin	C57BL/6J mouse			↓	Chalouhi et al. (2016)[27]
	Aspirin	C57BL/6J mouse			↓	Suzuki et al. (2018)[42]
COX-2	Celecoxib	Sprague–Dawley rat	↓	↓		Aoki et al. (2011)[20]
	NS-398	C57BL/6J mouse			↓	Chalouhi et al. (2016)[27]
Prostaglandin E receptor subtype 2 (EP2)	PF-04418948	Sprague–Dawley rat		↓		Aoki et al. (2017)[23]
Sphingosine-1 phosphate receptor type 1 ($S1P_1$)	ASP4058	Sprague–Dawley rat		↓		Yamamoto et al. (2017)[15]
Tumor necrosis factor (TNF) -α	Etanercept	Sprague–Dawley rat		↓		Yokoi et al. (2014)[48]
	3,6′dithiothalidomide	C57BL/6J mouse	↓		↓	Starke et al. (2014)[41]
Matrix metalloproteinases (MMPs)	Minocycline	C57BL/6J mouse			↓	Makino et al. (2012)[37]
	Doxycycline	C57BL/6J mouse			↓	Makino et al. (2012)[37]
	Tolylsam	Sprague–Dawley rat		↓		Aoki et al. (2007)[12]
	Imidapril	Sprague–Dawley rat		↓		Ishibashi et al. (2012)[32]
Inducible nitric oxide synthase (iNOS)	Aminoguanidine	Sprague–Dawley rat	↓			Fukuda et al. (2000)[21]
Endothelin receptor	K-8794	Sprague–Dawley rat		↓		Sadamasa et al. (2007)[38]
Cathepsins	NC-2300	Sprague–Dawley rat		↓		Aoki et al. (2008)[25]
Reactive oxygen species	Edaravone	Sprague–Dawley rat		↓		Aoki et al. (2009)[26]
	Edaravone	Japanese white rabbit		↓		Hu et al. (2018)[29]
Phosphodiesterase 4	Ibudilast	Sprague–Dawley rat		↓		Yagi et al. (2010)[47]
Rho-kinase	Fasudil hydrochloride	Sprague–Dawley rat	↓			Eldawoody et al. (2010)[28]
Peroxisome proliferator-activated receptor-γ (PPAR-γ)	Pioglitazone	C57BL/6J mouse			↓	Shimada et al. (2015)[39]
Dipeptidyl peptidase-4 (DPP-4)	Anagliptin	Sprague–Dawley rat		↓		Ikedo et al. (2017)[31]

(*Continued*)

Table 1 Potential therapeutic targets fot the treatment of intracranial aneurysms demonstrated in rodent models—Continued

Therapeutic target	Drug	Animal	Effect on aneurysmal pathogenesis			Author (year)
			Formation	Enlargement	Rupture	
Angiotensin-converting enzyme (ACE)	Captopril	C57BL/6J mouse			↓	Tada et al. (2014)[43]
Angiotensin II receptor type 1 (AT1)	Losartan	C57BL/6J mouse		↓		Tada et al. (2014)[43]
Angiotensin II receptor type 2 (AT2)	Angiotensin-(1-7)	C57BL/6J mouse			↓	Shimada et al. (2015)[40]
Mineralocorticoid receptor	Eplerenone	Sprague–Dawley rat	↓	↓		Tada et al. (2009)[44]
Estrogen receptor	17β-estradiol	C57BL/6J mouse			↓	Tada et al. (2009)[46]
	Diarylpropionitrile	C57BL/6J mouse			↓	Tada et al. (2009)[46]
	Diarylpropionitrile	C57BL/6J mouse	↓			Tada et al. (2009)[45]
	Bazedoxifene	Sprague–Dawley rat			↓	Maekawa et al. (2017)[36]
Mast cell	Emedastine difumarate	Sprague–Dawley rat		↓		Ishibashi et al. (2010)[30]
	Tranilast	Sprague–Dawley rat		↓		Ishibashi et al. (2010)[30]
Macrophage	Clodronate liposome	C57BL/6J mouse	↓			Kanematsu et al. (2011)[33]

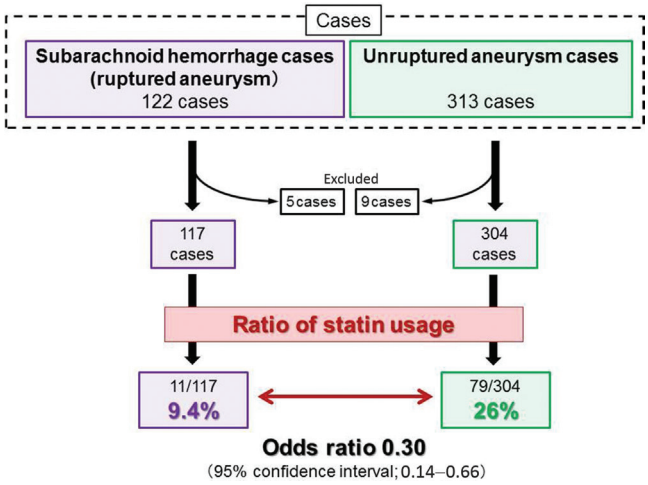

Fig. 1 Preventive effect of statins on the onset of subarachnoid hemorrhage by rupture of intracranial aneurysm (data from Yoshimura et al.[52]).

inhibitors; widely-used cholesterol lowering drugs) or non-steroidal anti-inflammatory drugs (NSAIDs) is inversely associated with SAH by rupture of IAs[49–52] (Fig. 1), supporting the notion that rupture of IAs can be prevented by a medical therapy in human cases. The intervention trial investigating the efficacy of statin (atrovastatin) usage in rupture, changes in shape or enlargement of small (<5 mm) unruptured IAs is on-going in Japan [SUAVe-PEGASUS trial (UMIN000005135)]. However, considering the prevalence of dyslipidemia in patients with IAs, only one-fourth, and the uncertainness of a long-term safety of low LDL level in serum, stains may be able to use only in limited cases. Furthermore, in secondary prevention for ischemic stroke, the usage of atorvastatin increases the incidence of hemorrhage stroke with the hazard ratio of 1.66 according to a prospective cohort study.[53] Thereby, statin usage to prevent rupture of SAH requires a careful attention.

Because of hemorrhagic diathesis by the anti-platelet effect, the application of NSAIDs as a pre-emptive medication to prevent SAH requires a careful evaluation. Indeed, while there are several case-control studies demonstrating inhibitory effect of NSAIDs on the onset of SAH,[51,54] another study demonstrates that the usage of NSAIDs increases the onset of SAH.[55] Because such an adverse effect of NSAIDs is derived from a non-selective inhibition of COX-1 responsible for the physiological production of prostaglandins and an inducible form of COX, COX-2, a selective antagonist acting on a specific receptor subtype functioning specifically in microenvironment of lesions may be ideal.[23] In this point of view, an antagonist specific for EP2 may become a powerful candidate. Here noted that the induction of COX-2 and EP2 in lesions makes EP2 antagonisms being specific for IA lesions.[23] The selective EP2 antagonist, PF-04418948, indeed effectively suppresses the enlargement of pre-existing IAs and also the degenerative changes in media in rats[23] (Fig. 2).

Potential therapeutic targets and candidates of drugs to prevent the disease revealed by animal studies are summarized in Table 1 and Fig. 3.

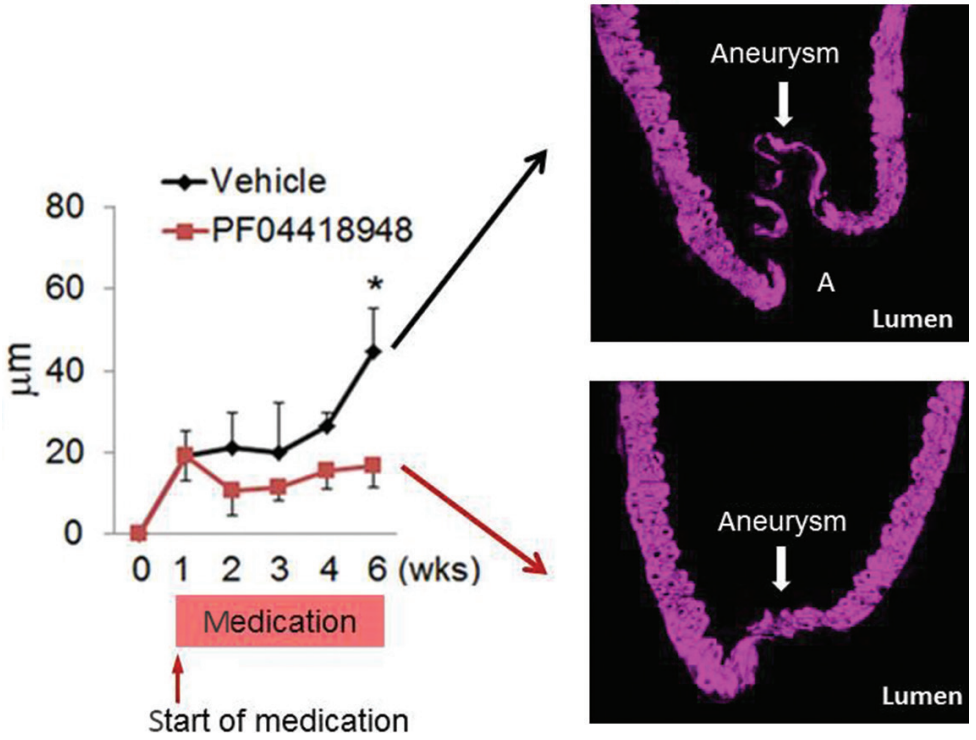

Fig. 2 Suppression of the enlargement of intracranial aneurysms and the degenerative changes of media by an oral administration of a selective EP2 antagonist, PF-04418948, in rats. Representative images of immunostaining for α-smooth muscle actin, a marker of medial smooth muscle cells, from vehicle- or PF compound–treated rats are shown. (Modified from Aoki et al.[23]).

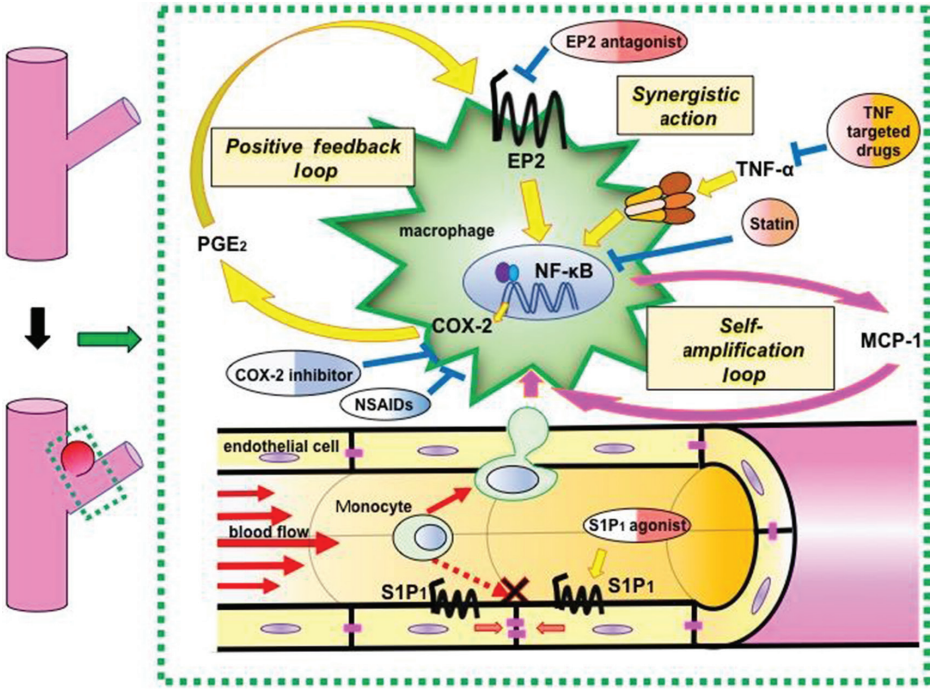

Fig. 3 Schematic drawing of potential therapeutic targets of intracranial aneurysms and drugs acting on these targets.

Conflicts of Interest Disclosure

All authors have no conflict of interest and registered online Self-reported COI Disclosure Statement Forms through the website for The Japan Neurosurgical Society.

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