NF-κB as a crucial factor for intracranial aneurysm formation and the potential of statins as drugs for intracranial aneurysm treatment through their anti-NF-κB effect

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Abstract

Introduction
Intracranial aneurysm (IA) is a regional bulging of intracranial arteries with degenerative changes in arterial walls. Because IA has a high incidence in general public and can cause subarachnoid haemorrhage, a most severe form of stroke with a high mortality rate, after rupture, treatment of IA is socially important. Nonetheless, to date, there is not any medical treatment available to prevent rupture except for surgical interventions with an unescapable risk of complication. Therefore, development of drugs for IA treatment based on mechanisms of IA formation is socially desired. In the present article, authors will summarize the recent advancement of researches toward the development of drugs for IA treatment.

Discussion
Findings from human studies and recent experimental studies using rodent models of IAs have proposed the notion that the chronic inflammation mediated by NF-κB activation regulates IA formation and also suggested the potential of NF-κB as therapeutic target for treatment. Indeed, orally administered HMG-CoA reductase inhibitors (statins) exert the excellent inhibitory effect on formation and enlargement of IAs in rodent models through their potent anti-NF-κB effect. Based on these findings, the hospital-based case-control study enrolling patients with unruptured or ruptured IAs to examine the effect of statin usage on rupture of IAs in human cases is recently carried out and beneficial effect of statins on rupture of IAs have been revealed. The ratio of statin usage between two groups is significant different and statistically statin usage reduces the risk of rupture of IAs at the adjusted odds ratio of 0.30, suggesting the potential of statins as candidate of drugs for IA treatment to prevent rupture.

Conclusion
Recent series of experimental and clinical studies greatly promote our understanding of mechanisms underlying IA formation and make a great advancement toward the development of drugs for IA treatment.

Introduction
Recent advance in medical technology greatly improve the treatment and outcome of a variety of cardiovascular diseases. However, current situation surrounding intracranial aneurysm (IA) is quite different and far from satisfactory as discussed in following. IA is a regional bulging of intracranial arteries mainly affected bifurcation sites and histologically characterized by excessive degenerative changes of arterial walls, disrupted internal elastic lamina and thinning of media. IA can cause subarachnoid haemorrhage after rupture though this lesion is asymptomatic before rupture in most cases. Because subarachnoid haemorrhage still poses a high mortality and morbidity rate despite intensive care and advancement of treatment and the prevalence of IA is high in general public (1 to 5 percent),1,3, treatment of IAs before rupture to prevent devastating subarachnoid haemorrhage is socially of significance. Indeed, in developed countries, many IAs are incidentally found before rupture through brain check or so. Nevertheless, even today, there is not any medical treatment available for such unruptured IAs to prevent rupture except for surgical procedures (microsurgical clipping and endovascular coiling)4. Given the unavoidable risk of complication associated with surgical procedures and the presence of many patients without surgical indications, such as elder ones or ones with comorbidity, a novel drug to prevent rupture of pre-existing IAs should be developed.

In the current review, we provide the brief summary regarding the contribution of NF-κB-mediated inflammation to IA formation and discuss the potential of NF-κB as a therapeutic target. Further, we refer to the future prospection of compounds with anti-NF-κB effect, statins, as drugs for IA treatment.

Discussion
The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies. Also, animal care was in accordance with the institution guidelines.

Crucial role of NF-κB-mediated inflammation in IA formation
To date, a large amount of studies using human IA specimen aimed to clarify
mechanisms of IA formation have been published and revealed the presence of active inflammatory responses in IA lesions, suggesting the role of inflammatory responses in IA formation. For example, expressions of pro-inflammatory mediators, such as TNF-κ, and infiltration of inflammatory cells in lesions especially macrophages are detected5,6,7,8,9.

Further, gene expression profile analyses have revealed the induction of inflammatory cascade, such as antigen processing, in lesions and suggest the involvement of inflammatory responses in IA formation10 and also in rupture of IAs, in the latter through bioinformatics analysis of comprehensive gene expressions NF-κB is identified as a regulatory transcription factor of induced genes in lesions and as a possible factor related with rupture11.

Consistently with findings from human studies described above, recent experimental studies mainly using animal models of IAs12,13 have clarified the involvement of long-lasting (chronic) inflammation in the pathogenesis of IA formation and progression14,15,16,17,18,19,20. In a rodent model, IAs are induced through increase of haemodynamic stress at bifurcation sites of intracranial arteries, a prospective site of IA formation in human cases, by one side of carotid ligation and induced hypertension by salt over-loading12,13.

Because IAs induced in this model share common pathological features with human ones, such as disrupted internal elastic lamina and degenerative changes of media, and also spontaneously rupture like in humans, this model is considered to mimic human disease well12,13. In a series of studies using these rodent models, the crucial role of chronic inflammation in IA formation and the significant contribution of NF-κB activation to the regulation of such inflammatory responses have been clarified16,21.

Here, NF-κB is a transcription factor activated in response to outward stimuli and induces expressions of various pro-inflammatory genes as a master regulator under inflammatory settings. In IAs, some of NF-κB-regulated genes, such as IL-1β (22), matrix metalloproteinase (MMP-9)15, cyclooxygenase-2 (COX-2)17 and monocyte chemoattractant protein-1 (MCP-1)14,18 are induced and contribute to the initiation and maintenance of inflammatory responses regulating IA formation and progression (Figure 1). NF-κB is activated during IA formation in endothelial cells of intracranial arteries presumably through high wall shear stress16, a putative trigger of IA formation23, loaded on bifurcation sites of intracranial arteries and also in macrophages, major inflammatory cells present in IA walls, recruited in lesions via their chemoattractant, MCP-114,18 (Figure 1).

NF-κB, activated in these types of cells, transcriptionally induces various pro-inflammatory mediators such as cytokines like IL-1β and proteinases like MMP-9 and exacerbates inflammation in IA lesions16.

Importantly, inflammation is not regressed in a short time like acute inflammation but rather lasting a long-period, sometimes years in humans. Recent findings have revealed the contribution of NF-κB activation also in this process17. NF-κB, activated in endothelial cells under hemodynamic stress, transcriptionally induces prostaglandin-producing enzyme, COX-2, in these cells and COX-2 produces prostaglandin E2.

Produced prostaglandin E2, then, activates NF-κB through acting on one of its receptor subtype, EP217. As a consequence, under haemodynamic stress, positive feedback loop consisting of COX-2-prostaglandinE2-EP2-NF-κB is formed (Figure 1). This means that, once haemodynamic stress drives this cascade, inflammatory response is amplified and becomes chronic through forming an amplification loop by a positive feedback mechanism17. The critical contribution of NF-κB to IA formation and progression is definitely shown in a recent publication. In this report, deficiency of NF-κB p50 subunit in mice inhibits IA formation to the level in wild type mice and, consistently, inhibition of NF-κB transcriptional activity by decoy oligonucleotides in rats significantly suppresses enlargement of

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IAs through inhibiting inflammatory responses in lesions\textsuperscript{16}. These findings from human specimen and animal models have supported the notion that IA is a NF-κB-mediated chronic inflammatory disease in intracranial arteries and proposed the potential of NF-κB as a therapeutic target for treatment.

**Stains as potential therapeutic drugs for IA treatment (prospection from experimental studies)**

As described in the previous section, recent findings regarding the mechanisms underlying IA formation have proposed the potential of drugs with anti-NF-κB effect as ones for IA treatment\textsuperscript{23}.

Hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, generally well-known as statin, is a cholesterol-lowering drug widely used worldwide. In addition to its excellent cholesterol-lowering effect, statin has the potent anti-inflammatory effect especially anti-NF-κB effect, known as "pleiotropic effect of statins". Though a precise mechanism of its anti-NF-κB effect is not clear, it is presumed that the lipid modifications such as prenylation by intermediates in the formation of cholesterol from Acetyl-CoA, such as geranyl pyrophosphate/geranylgeranyl pyrophosphate, are essential for various proteins to perform their functions and the pharmacological inhibition of HMG-CoA reductase (it metabolizes Acetyl-CoA to Mevalonate) by statins blocks this process\textsuperscript{24}.

In IAs, the inhibitory effect of statins on IA formation and progression have been clarified in recent studies, in which the orally administered statins to rat model effectively suppress the incidence or further enlargement of IAs through inhibiting NF-κB-mediated inflammatory responses in lesions\textsuperscript{25,26,27} though only one report demonstrates the deleterious effect on IA progression\textsuperscript{28} (Figure 1).

In these studies, three kinds of statins orally administered to rat model of IAs, pravastatin\textsuperscript{25}, simvastatin\textsuperscript{26} or pitavastatin\textsuperscript{27}, all significantly suppress the formation, progression or further enlargement of induced IAs independent of their cholesterol-lowering effects. The inhibitory effect of statins on IAs, therefore, seems to be not restricted in a specific kind of statins but a common feature of statins.

In IA lesions treated with orally administered statins, inflammatory responses, including NF-κB activation in aneurysm walls, subsequent expressions of pro-inflammatory mediators such as MCP-1 and macrophage infiltration via MCP-1, are significantly suppressed through the pleiotropic effect of statins as expected\textsuperscript{26,27} (Figure 1).

One report shows the promotion of IA progression in accompanied with the acceleration of apoptotic cell death in IA walls under treatment with 'high dose' pravastatin or simvastatin in rat models\textsuperscript{28}, in complete opposite from results of other studies\textsuperscript{25,26,27}. Because, in this report, pravastatin dose-dependently exhibits anti-inflammatory effect in cultured endothelial cells, induction of eNOS expression and suppression of ICAM-1 expression, and consistently reduces endothelial cell damage in intracranial arteries in vivo at 'low dose'\textsuperscript{29}, the deleterious effect of pravastatin and simvastatin may be due to the dosage in lesions. Further, usage of different sexes in rat models used in these studies, male in\textsuperscript{26,27} and female in\textsuperscript{28}, may influence the results.

Given the established safety of statins in humans based on the data from many clinical studies including great many patients and the excellent inhibitory effect on IA progression demonstrated in rat models, statins may be promising drugs for IA treatment to prevent the further enlargement and rupture of pre-existing IAs in patients.

**Future prospection of statins as drugs for IA treatment**

Studies addressing the effect of statins on IAs in human cases are still quite limited\textsuperscript{29,30}. Therefore, the question, whether statins can be therapeutic drugs for IA treatment to prevent rupture and resultant subarachnoid haemorrhage, is left open and further well-organized clinical studies are desired. In this section, we will briefly summarize the published reports concerning the effect of statins on IAs in human cases and discuss the potential of statins as drugs for treatment.

One single-centre case-control study demonstrates the independence of the incidence of IAs from statin usage\textsuperscript{29}. A total of 300 cases (patient with IA) and 900 controls are enrolled in this study and the correlation of statin usage with the presence of IA is analysed\textsuperscript{29}. Because, in this case-control study, established risk factors for IA development, hypertension and smoking\textsuperscript{1}, are picked up as factors positively correlated with the presence of IAs with the odds ratio of 4.02 and 1.67, respectively\textsuperscript{29}, and the results obtained are authoritative. As statin usage is not correlated with the incidence of IAs from this study\textsuperscript{29}, statins may not influence IA formation.

Rupture of pre-existing IAs is, of course, the most serious concern in society due to the severity of consequent subarachnoid haemorrhage\textsuperscript{2}. Further, patients with incidentally found IAs are main subjects for treatment. Therefore, the effect of statins on pre-existing IAs in patients, not the incidence of IAs, should be examined. Only one hospital-based case-control study recently published has addressed this point and implicated the potential of statins as drugs to prevent rupture\textsuperscript{30}.

In this case-control study, 117 cases (patients with ruptured IAs (patients affected with subarachnoid haemorrhage) and 304 controls (patients with unruptured IAs) are enrolled from 15 institutes in Japan\textsuperscript{30}.

Background characteristics of cases or controls enrolled in the study are similar\textsuperscript{30}. As 'size of IAs' and 'current smoking' are appropriately picked up as factors correlated with rupture as expected, the results obtained from this study seems to be reliable. In this study, rate of statin usage is remarkably different between groups, 9.4% in cases (11/117) and 26.0% in controls (79/304), respectively, and difference reaches statistically significant (p<0.001)\textsuperscript{30}.

Further, after stratifying the data according to serum cholesterol level,
statin usage still inversely correlates with the risk of rupture in patients with serum cholesterol level of over 130mg/dL. In logistic regression analysis, the usage of any statin, regardless the kind of statins, is inversely correlated with rupture at the adjusted odds ratio of 0.30. Based on findings from the series of studies, the potential of statins as therapeutic drugs for preventing rupture of pre-existing IAs has been proposed. Statins can be a good candidate of drugs for IA treatment in patients with unruptured IAs and concomitant hypercholesterolemia. However, because statins have quite a potent cholesterol-lowering effect and can decrease serum cholesterol level even in healthy person without hypercholesterolemia, resulting in cholesterol level below the normal limit in those patients, the safety of statins on patients without hypercholesterolemia should be established to warrant further randomized placebo-controlled study. Here, noted that a major part of patient with IAs (about 74% from our study) is without hypercholesterolemia.

Conclusion
Recent experimental findings have addressed the importance of NF-κB-mediated chronic inflammation in IA formation and proposed NF-κB as a potential therapeutic target for IA treatment. Based on these findings, statins with a potent anti-NF-κB effect have been applied to rodent models and their inhibitory effect on IAs has been demonstrated. Further, recent case-control study clarifies the inhibitory effect of statins on rupture of pre-existing IAs in patients suggesting the potential of these drugs as promising ones for IA treatment. Recent researches have greatly advanced our understanding of IAs and became the development of medical treatment of IAs more likely.

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References


