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Protective role of peroxisome proliferator-activated receptor- γ in the development of intracranial aneurysm rupture

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Abstract

Background and Purpose—Inflammation is emerging as a key component of the pathophysiology of intracranial aneurysms. Peroxisome proliferator-activated receptor- γ (PPAR γ) is a nuclear hormone receptor of which activation modulates various aspects of inflammation.

Methods—Using a mouse model of intracranial aneurysm, we examined the potential roles of PPAR γ in the development of rupture of intracranial aneurysm.

Results—A PPAR γ agonist, pioglitazone (PGZ), significantly reduced the incidence of ruptured aneurysms and the rupture rate without affecting the total incidence aneurysm (unruptured aneurysms and ruptured aneurysms). PPAR γ antagonist (GW9662) abolished the protective effect of PGZ. The protective effect of PGZ was absent in mice lacking macrophage PPAR γ . PGZ treatment reduced mRNA levels of inflammatory cytokines (monocyte chemoattractant factor-1, interleukin-1, and interleukin-6) that are primarily produced by macrophages in the cerebral arteries. PGZ treatment reduced the infiltration of M1 macrophage into the cerebral arteries and the macrophage M1/M2 ratio. Depletion of macrophages significantly reduced the rupture rate.

Conclusion—Our data showed that the activation of macrophage PPAR γ protects against the development of aneurysmal rupture. PPAR γ in inflammatory cells may be a potential therapeutic target for the prevention of aneurysmal rupture.

Keywords

intracranial aneurysm; inflammation; subarachnoid hemorrhage

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Conflict(s) of Interest/Disclosure(s)

None

Introduction

Subarachnoid hemorrhage from the rupture of intracranial aneurysm can result in significant adverse consequences. The 30-day mortality rate after aneurysmal subarachnoid hemorrhage is as high as 45%.¹ Surgical clipping or endovascular coiling can be offered to patients with unruptured aneurysms for the prevention of aneurysmal rupture. Significant technical advancements and refinements have been made in these invasive treatments. However, the mortality and morbidity resulting from the clipping and coiling of unruptured aneurysms are not negligible.² For the further improvement of these therapies, better understanding of the pathophysiology of intracranial aneurysms is needed.

Inflammation is emerging as a key component of the pathophysiology of intracranial aneurysms. Inhibition and modulation of inflammation or inflammatory cytokines have been proposed as a therapy to modify the natural course of intracranial aneurysms and prevent the development of aneurysmal subarachnoid hemorrhage.^{3, 4} Peroxisome proliferator-activated receptor- γ (PPAR γ) is a nuclear hormone receptor of which activation modulates various aspects of inflammation. PPAR γ is expressed in many cell types, including endothelial cells, smooth muscle cells and inflammatory cells, cell types that are important for the pathophysiology of intracranial aneurysms.⁵ Activation of PPAR γ reduces inflammation through the modulation of the production and activation of inflammatory cytokines that have been linked to the pathophysiology of intracranial aneurysms.^{6–8} Therefore, PPAR γ may play pivotal roles in the progression and rupture of intracranial aneurysms by modulating inflammation. In this study, using a mouse model of intracranial aneurysm, we examined the potential roles of PPAR γ in the development of aneurysmal rupture that results in subarachnoid hemorrhage.

Material and Methods

Experiments were conducted in accordance with the guidelines approved by the University of California, San Francisco, Institutional Animal Care and Use Committee. We combined induced systemic hypertension (deoxycorticosterone acetate-salt hypertension) and a single injection of elastase into the cerebrospinal fluid at the right basal cistern as previously described.^{4, 9, 10}

To detect aneurysmal rupture, two blinded observers performed daily neurological examination as previously described.⁴ Neurological symptoms were scored as follows: 0: normal function; 1: reduced eating or drinking activity demonstrated by a weight loss greater than two grams of body weight (approximately 10% weight loss) over 24 hours; 2: flexion of the torso and forelimbs upon lifting the whole animal by the tail; 3: circling to one side with a normal posture at rest; 4: leaning to one side at rest; and 5: no spontaneous activity. When mice were found to show neurological symptoms associated with aneurysmal rupture (neurological score: 1–5),⁴ they were euthanized immediately (within 4 hours). Since our previous studies using this model showed that aneurysmal rupture occur within three weeks of aneurysm induction,^{4, 5, 11, 12} asymptomatic mice were euthanized 21 days after aneurysm induction as previously described.^{11,12}

The brain samples were perfused with phosphate-buffered saline, followed by a gelatin containing blue dye to visualize cerebral arteries. Aneurysms were defined as a localized outward bulging of the vascular wall, whose diameter was greater than the parent artery diameter.^{9, 10}

Our preliminary studies found that aneurysmal rupture can be found as early as 6 days after the aneurysm induction in this model and that aneurysmal formation occurs first 6 days after aneurysm induction.⁵ Therefore, in this study, the treatments with pioglitazone (PGZ, PPAR γ agonist, 10 mg/kg/day¹³) or GW9662 (PPAR γ antagonist, 2 mg/kg/day¹³, or both of them were started 6 days after aneurysm induction and continued until 3 weeks (21 days) after aneurysm induction.^{4, 11, 12} This timing of the treatment was found to affect aneurysmal rupture without affecting the formation of aneurysms.^{4, 11, 12} PPAR γ agonist and GW9662 were separately dissolved in 20% dimethyl sulfoxide (DMSO), and then were injected to the mice intraperitoneally once a day. Twenty percent DMSO was used in the vehicle control group. To detect any unexpected side effects from the drug treatment, we measured systolic blood pressure every week and body weight and general appearance daily.

Macrophage depletion was achieved by an intravenous injection of liposome-encapsulated dichloromethylene diphosphonate (clodronate liposome).^{10, 14, 15} Mice received clodronate liposomes intravenously 6 days after aneurysm induction. Our previous studies showed that this regimen was reported to cause a reduction of macrophages to less than 10% of baseline count.^{10, 15} Animals in the control group received the same volume of phosphate-buffered saline-containing liposome (PBS liposome).

We obtained PPAR γ flox/flox mice¹⁶ and mice expressing Cre recombinase under the control of the myeloid-specific lysosome M (LysM) promoter from Jackson Laboratory (LysMCre)¹⁷ (Bar Harbor, Maine, U.S.A.). The appearance, growth and body weight of macrophage/granulocyte specific PPAR γ knockout mice were normal. We used LysMCre-negative PPAR γ flox/flox mice as a control and LysMCre-positive PPAR γ flox/flox mice as macrophage/granulocyte specific PPAR γ knock out mice.

For the real-time PCR analysis, the total RNA was collected at 14 days after aneurysm induction from the mice that were dedicated for the mRNA analysis. Since our previous studies showed that aneurysmal rupture in this model occurs between 6 days and 21 days after aneurysm induction,^{4, 11, 12} we have chosen 14 days after aneurysm induction as a mid-point for detecting mRNA changes associated with aneurysmal rupture. We collected the control cerebral arteries, aneurysm tissues from mice that were treated with PGZ, and aneurysms tissues from mice that were treated with vehicle (n = 8 for each group) We assessed mRNA expression of monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-1 β (IL-1 β), inducible nitric oxide synthase (iNOS), and matrix metalloproteinase-9 (MMP-9). The total RNA was extracted using the RNeasy Mini Kit (Qiagen, CA, USA). The total RNAs were transcribed to cDNA using QuantiTect reverse transcription kit (Qiagen). The mRNA expression levels were determined using SYBR Green technology (Applied Biosystems, CA, USA). Quantitative values were obtained from the threshold cycle value (Ct) and the data were analyzed by the 2^{-CT} method. The transcript amount of GAPDH (glyceraldehyde-3-phosphate

dehydrogenase) was quantified as an internal RNA control. The expression levels were normalized with the mean expression level in the normal cerebral artery as 1.0.

For quantitative analysis of macrophage infiltration and macrophage phenotype, we collected the brain tissue samples from the mice treated with vehicle and from the mice treated with PGZ at 14 days after aneurysm induction (n = 6 from each group). These mice were dedicated for the Immunohistochemical analyses. The brain tissues were frozen in optimal cutting temperature compound and were sectioned near middle cerebral artery (MCA). Immunohistochemistry with antibodies for M1 (IL-12 p40, GeneTex, Irvine, CA), M2 (CD206, AbD Serotec, Raleigh, NC), macrophage (CD68, Bio Rad, Hercules, CA) and macrophage-mediated phagocytosis (CD36, abcam, Cambridge, MA) were performed to the brain tissue sections. We chose the field with the highest number of cells around MCA for each sample and quantified the number of the infiltrated cells by cell counting (positive immunostained cells) per high-power field (HPF) (40X).

Statistical analysis

All of the results were expressed as mean $\pm$ SD. Fisher's exact test was used to analyze the incidence of aneurysms (number of mice with any aneurysms (ruptured or unruptured) / total number of mice), the incidence of ruptured aneurysms (number of mice with ruptured aneurysms / total number of mice) and rupture rate (number of mice with ruptured aneurysms / number of mice with any aneurysms). Mice that did not show aneurysm formation were excluded from the calculation of the rupture rate. As an exploratory analysis, the survival analysis was performed using the Log-rank test. Mice that did not develop aneurysms were excluded in the survival analysis. Levels of mRNA were compared between two groups using Mann-Whitney test. Statistical significance was accepted at $P < 0.05$. GraphPad Prism 6 was used for statistics software.

Results

PPAR γ agonist was protective against the development of intracranial aneurysm rupture

As a first step to investigate the roles of PPAR γ in the development of intracranial aneurysmal rupture, we treated the 8–10 weeks-old male wild-type mice (C57BL/6J, The Jackson Laboratory, Maine) with a PPAR γ agonist, pioglitazone (PGZ), starting at 6 days after aneurysm induction (i.e., after aneurysm formation) and the treatments were continued until 21 days after aneurysm induction or until mice developed aneurysmal rupture, whichever came earlier.

There was no significant difference in the incidence of aneurysms between vehicle and PGZ treatment groups (84 vs. 88%, n = 19 vs. 17) (Figure 1A). However, compared to the vehicle treatment, the PGZ significantly reduced the incidence of ruptured aneurysms (Figure 1A; vehicle control vs. PGZ: 68 vs. 29%, $P < 0.05$) and the rupture rate (Figure 1B; vehicle control vs. PGZ: 81 vs. 33 %, $P < 0.05$, n = 16 vs. 15). For the purpose of exploratory analysis, a symptom-free curve (Kaplan-Meier analysis curve) was plotted after excluding mice that did not have aneurysms (Figure 1C). A log-rank test revealed a significant reduction of aneurysmal rupture with the PGZ ($P < 0.05$). Figure 2 shows representative

unruptured aneurysm (B) and ruptured aneurysm (C). To visualize the ruptured aneurysm that was covered by the blood clots from subarachnoid hemorrhage, we have removed most of the blood clots (Figure 2C). The picture was taken after the removal blood clots (Figure 2C).

To further confirm the protective effect of PPAR γ activation against the development of aneurysmal rupture, we added the PPAR γ antagonist (GW9662) to PGZ treatment group. The incidence of ruptured aneurysms and rupture rate was normalized by the treatment with GW9662 (Figures 1A and 1B, incidence of ruptured aneurysms; PGZ vs. PGZ + GW9662 29 vs. 62%, $P < 0.05$, $n = 17$ vs. 16; rupture rate; PGZ vs. GW9662 33 vs. 77%, $P < 0.05$; $n = 15$ vs. 13). GW9662 did not have any significant effect on the incidence of ruptured aneurysms and rupture rate when used alone. There was no significant difference in the blood pressure, body weight, and blood sugar levels between PGZ-treated mice and the vehicle-treated mice (Supplemental Figure and Table). In addition, we did not observe any apparent cardiovascular complications or fluid retention.

Activation of PPAR γ reduced inflammatory molecules in the cerebral arteries

We examined the mRNA expression of inflammatory molecules in the cerebral arteries and intracranial aneurysms using real-time PCR. Expression levels of IL-6, IL-1 β , and MCP-1 were significantly lower in the aneurysm tissues collected from PGZ-treated mice ($n = 8$) than in those from vehicle-treated mice ($n = 8$) (IL-6: 4.9 ± 2.9 vs. 53.8 ± 66.2 ; IL-1 β : 17.3 ± 17.2 vs. 37.6 ± 20.8 ; MCP-1: 3.8 ± 1.3 vs. 6.2 ± 1.7 ; all $P < 0.05$) (Figure 3A). As a post-hoc analysis, we found that the expression levels of TNF- α , MCP-1, iNOS, IL-1 β , and IL-6 were increased in the aneurysm tissues from vehicle-treated mice compared to control cerebral artery samples ($n = 8$) (TNF- α : 4.8 ± 1.0 vs. 1.0 ± 0.5 ; MCP-1: 6.2 ± 1.7 vs. 1.0 ± 0.6 ; iNOS: 3.5 ± 2.5 vs. 1.0 ± 1.0 ; IL-1 β : 37.6 ± 20.8 vs. 1.0 ± 0.3 ; IL-6: 53.8 ± 66.2 vs. 1.0 ± 1.0 , all $P < 0.05$). There was a trend that the expression level of MMP-9 was lower in PGZ-treated mice than in vehicle-treated mice (4.24 ± 3.5 vs. 6.76 ± 7.4 , $P = 0.35$) (Figure 3A).

Depletion of macrophages after aneurysm formation reduced the incidence of ruptured aneurysm and rupture rate

Since the activation of PPAR γ by PGZ reduced the expression of inflammatory molecules that are mainly expressed in macrophages, we examined whether macrophages were required for the development of aneurysmal rupture. Macrophages were depleted by the treatment of clodronate liposome after aneurysmal formation. The control group received the PBS liposome.

Macrophage depletion by the clodronate liposome significantly reduced the incidence of aneurysmal rupture and rupture rate (Figures 4A and 4B, incidence of ruptured aneurysms; PBS liposome vs. clodronate liposome 65 vs. 23%, $P < 0.05$, rupture rate; PBS liposome vs. clodronate liposome 76 vs. 29%, $P < 0.05$, $n = 23$ vs. 22). The survival analysis also showed that mice treated with clodronate liposome significantly improved the symptom free survival rate compared to mice treated with PBS liposome (Figure 4C, $P < 0.05$, $n = 19$ vs. 19). There was no significant difference in blood pressure between two groups (Supplemental Figure).

Macrophage / granulocyte PPAR γ was required for the protective effect of PGZ against the development of aneurysmal rupture

To test whether the protective effect of PPAR γ activation against the development of aneurysmal rupture requires PPAR γ in inflammatory cells, we compared the incidence of aneurysmal rupture between macrophage/granulocyte-specific PPAR γ knockout (PPAR γ flox/flox LysMCre $^{+/-}$ or PPAR γ flox/flox LysMCre $^{+/+}$, n = 20) and the control mice (PPAR γ flox/flox LysMCre $^{-/-}$, n = 20).

There was no significant difference in the overall incidence of aneurysm between macrophage/granulocyte-specific PPAR γ knockout mice and the control mice (85 vs. 87%, n = 20 vs. 15) (Figure 5A). There was no difference in blood pressure between macrophage/granulocyte-specific PPAR γ knockout mice and the control mice (Supplemental Figure).

The activation of PPAR γ by PGZ significantly decreased the incidence of ruptured aneurysms and rupture rate in the LysMCre-negative PPAR γ flox/flox mice (control mice) (incidence of ruptured aneurysms VC vs. PGZ = 70 vs. 30%, $P < 0.05$; rupture rate: VC vs. PGZ = 82 vs. 35%, $P < 0.05$, Figure 5A, 5B). However, the protective effect of PGZ was absent in LysMCre-positive PPAR γ flox/flox mice (macrophage specific PPAR γ knockout mice), as there was no difference the incidence of ruptured aneurysm or rupture rate between the LysMCre-positive PPAR γ flox/flox mice treated with the vehicle and the LysMCre-positive PPAR γ flox/flox mice treated with PGZ (incidence of ruptured aneurysms VC vs. PGZ = 67 vs. 60%, $P = 1.0$, n = 20 vs. 15; rupture rate: VC vs. PGZ = 77 vs. 69%, $P = 1.0$, n = 17 vs. 13) (Figure 5A and 5B). The survival analysis using those mice that had aneurysms revealed a significant improvement of the survival by PGZ treatment in the LysMCre-negative PPAR γ flox/flox mice, but not in the LysMCre-positive PPAR γ flox/flox mice ($P < 0.05$) (Figure 5C).

Expression of PPAR γ in mouse intracranial aneurysms

To confirm expression of PPAR γ in aneurysms, we assessed expression and localization of PPAR γ by the real-time RT-PCR and immunohistochemistry, respectively. Immunohistochemical analysis showed that PPAR γ was expressed mainly in the adventitial layer of aneurysm where abundant macrophages (CD68 positive cells) were observed (Figure 3B, **left panel**). Aneurysm tissues expressed PPAR γ mRNA, at least, at the similar degree to the normal cerebral arteries ($P = 0.13$, Figure 3B, **right panel**)

Quantification of macrophage infiltration and macrophage phenotype

One mouse from PGZ treatment group was excluded because of intraoperative mortality. The number of IL12 p40 positive cell (M1) and CD68 positive cell were significantly lower in the collected tissue from PGZ-treated mice (n = 5) than in those from vehicle control group (n = 6) (IL12 p40: 4 ± 1.9 vs. 0.2 ± 0.4 , $P < 0.01$; CD68: 14.2 ± 7.9 vs. 33.2 ± 6.4 , $P = 0.01$). The ratio of M1 and M2 (M1/M2) was also lower in the collected tissue from PGZ-treated mice (0.01 ± 0.02 vs. 0.16 ± 0.1 , $P < 0.01$) (Figure 6A). There was no significant difference in the number of CD36 positive cells between PGZ-treated mice and vehicle-treated mice ($P = 0.57$) (Figure 6A). Figure 6B shows representative staining of the cerebral arteries for IL12 p40, CD68, CD36, and CD206.

Discussion

In this study, we sought to study the potential role of PPAR γ in the pathophysiology of intracranial aneurysms. Our data showed that the activation of PPAR γ in macrophages and possibly granulocytes protects against the development of aneurysmal rupture. In the animal model of intracranial aneurysm, a PPAR γ agonist, PGZ (pioglitazone), reduced the rupture rate. The protective effect of PGZ appears to require macrophage/granulocyte PPAR γ . Interestingly, a lack of macrophage/granulocyte PPAR γ did not affect the formation of aneurysms, indicating the unique role of PPAR γ in the development of aneurysmal rupture. In addition, the depletion of macrophages prevented aneurysmal rupture, further confirming the key role of macrophages in the development of aneurysmal rupture. Inflammatory cells or inflammatory cell PPAR γ may be a potential therapeutic target for the prevention of aneurysmal rupture.

Jones *et al.* showed the protective role of PPAR γ against the development and rupture of aortic aneurysms in Angiotensin II-treated apolipoprotein E (ApoE) knockout mice.⁶ Although both aortic aneurysm and intracranial aneurysm are morphologically similar, the underlying pathology and mechanisms are different between the two types of aneurysms. Atherosclerosis is considered as a key pathological event that leads to aortic aneurysm formation, and angiotensin II treatment of ApoE knockout mice causes atherosclerosis and aortic aneurysm formation simultaneously.¹⁸ In contrast, intracranial aneurysm formation in human is not associated with atherosclerosis, and histologically, intracranial aneurysms or their parent arteries are free from atherosclerotic changes.¹⁹ Despite different underlying pathologies among these two types of aneurysms, findings that activation of PPAR γ protected against the development of their ruptures may indicate that the mechanisms for the development of aneurysmal rupture may be similar between the types of aneurysms. Some of the proposed strategies of the pharmacological prevention of the rupture of aortic aneurysms may be applied to intracranial aneurysms.²⁰ For example, the treatment with PPAR γ agonists including thiazolidinediones, rosiglitazone, and pioglitazone has been proposed for aortic aneurysms.^{6, 21}

PPAR γ modulates inflammation by affecting the activation of various genes.^{22, 23} Activation of PPAR γ is known to reduce the elaboration of inflammatory cytokines from monocyte/macrophages.²⁴ Consistent with reports by others, we found the reduction of macrophage-related cytokines including IL-1, IL-6 and MCP-1 by the activation of PPAR γ .^{23–26} Previous studies that used animal models strongly suggest that excessive and sustained inflammation leads to the progression and rupture of intracranial aneurysms.^{4, 27, 28} Anti-inflammation agents prevented aneurysmal rupture in mice.⁴ Clinically, the use of anti-inflammatory agent was associated with the reduced risk of aneurysmal rupture in humans.³ Anti-inflammatory therapy is emerging as a potential therapy for prevention of aneurysmal rupture.²⁹ As a therapeutic target for modulating inflammation for the prevention of aneurysmal rupture, PPAR γ may be an attractive target since it mediate expression of many inflammation related genes and control inflammation at multiple-levels rather than affecting a single molecule or single pathway.²⁶ Moreover, there are clinically available PPAR γ activators including PZG. Although we have not fully investigated in this study, there may be additional mechanisms that are responsible for the

protective effect of PPAR γ activation. Such mechanisms may include the effects on matrix metalloproteinase activation, superoxide production and expression of angiotensin II receptors.^{23, 26}

In our study, the protective effect of PPAR γ activation against the development of aneurysmal rupture required macrophage PPAR γ . The similarly protective role of macrophage PPAR γ was observed in the animal model of atherosclerosis.³⁰ It should be noted that a lack of macrophage PPAR γ did not affect the formation of aneurysms in our study. Inflammation may play different roles between the formation of aneurysm and the development of aneurysmal rupture. While it is often assumed that there may be shared mechanisms between these two biological processes (i.e., aneurysm formation and aneurysmal rupture), underlying mechanisms may be fundamentally different between these two events. Further studies are needed to elucidate the underlying mechanisms that governing these processes.

Clinical studies strongly suggest roles of macrophage activation in the development of aneurysmal rupture.^{31, 32} CD163, a macrophage scavenger marker, was found to be significantly higher in ruptured intracranial aneurysm compared to unruptured aneurysms.³¹ Moreover, ruptured aneurysms had the higher ratio of M1/M2 macrophage than unruptured aneurysms, suggesting the role of the imbalance of macrophage polarization in the development intracranial aneurysmal rupture.³² In our study, the infiltration of macrophages was lowered by PGZ treatment. In addition, the PGZ treatment reduced the M1/M2 ratio. Our data suggest a causal relationship between M1 macrophages and the development of aneurysmal rupture.

There are a number of factors that can potentially limit the translational potential of our findings. First, the animal models do not completely replicate biological events that lead to aneurysm formation and growth. Aneurysms were induced, but not spontaneously formed, in these models. However, the phenotype and presentation of intracranial aneurysms in our model closely mimic those of human intracranial aneurysms.^{9, 10} More importantly, human intracranial aneurysms and aneurysms in this model share the common end phenotype, i.e., aneurysmal rupture and associated symptoms.^{4, 11} Future clinical studies that assess the correlation between aneurysmal rupture and the use of agents that affect PPAR γ activity will be needed to validate our findings. Second, the therapy targeting PPAR γ may affect the homeostatic regulation of metabolism through the modulation of PPAR γ activity in the adipose tissues. However, in our study, we did not find any significant effects of PGZ or a lack of macrophage PPAR γ on body weight or blood sugar levels, consistent with a previous study that used a mouse model of stroke.³³ Third, although our data indicate that the activation of PPAR γ affected the development of aneurysmal rupture through the modulation of pro-inflammatory cytokines, the precise underlying mechanism is not clear.

Summary

Our findings suggest that PPAR γ in inflammatory cells play an important role in the development of aneurysmal rupture in mice. PPAR γ may serve as a therapeutic target for the prevention of intracranial aneurysmal rupture.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Effects of pioglitazone on the development of aneurysmal rupture in male wild-type mice. Twenty mice for each group underwent the aneurysm induction. One mouse from vehicle control group, 3 mice from PGZ group, 4 mice from PGZ & GW9662 group and six mice from GW9662 group were excluded from the study because of intraoperative mortality. A PPAR γ agonist, pioglitazone (PGZ), reduced the incidence of ruptured aneurysm and rupture rate. A PPAR γ antagonist (GW9662) abolished the protective effect of PGZ. A: incidence of aneurysm; B: rupture rate; C: symptom-free curve (Kaplan-Meier analysis curve). Aneurysmal rupture in each group began to occur 7 days after the aneurysm induction. Mice that did not have aneurysms were excluded from this analysis. VC: vehicle control

Figure 2.

Representative aneurysms. A: no aneurysm; B: unruptured aneurysms; C: ruptured aneurysm. In the picture C, aneurysm formations were found after the removal of the blood clot from subarachnoid hemorrhage that was covering the right anterior portion of the Circle of Willis. The locations of aneurysms are indicated by rectangles.

Figure 3.

(A) Effects of PPAR γ activation by pioglitazone on mRNA levels of inflammatory cytokines. Expression levels of tumor necrosis factor- α (TNF- α), monocyte chemoattractant factor-1 (MCP-1), inducible nitric oxide synthase (iNOS), interleukin-1 (IL-1), and interleukin-6 (IL-6) were increased in aneurysm samples compared to control cerebral artery samples. Activation of PPAR γ by PGZ significantly reduced the expression levels of MCP-1, IL-1 and IL-6 in aneurysm tissues. VC: vehicle control (B) Expression of PPAR γ in mouse intracranial aneurysms. Left panel: In aneurysm tissues, PPAR γ was localized to the adventitial layer where macrophages (CD68) were abundant. Right panel: Aneurysm tissues expressed PPAR γ mRNA, at least, at the similar degree to the normal cerebral arteries.

Figure 4.

Depletion of macrophages after aneurysm formation reduced the incidence of ruptured aneurysm and rupture rate. Twenty-five mice for each group underwent the aneurysm induction. Two mice from PBS liposome group and three mice from clodronate liposome group were excluded from the analysis because of intraoperative mortality. Macrophage depletion by the clodronate liposome significantly reduced the incidence of aneurysmal rupture and rupture rate. C: symptom-free curve. Aneurysmal rupture in each group began to occur 7 days after the aneurysm induction. The survival analysis also showed that mice treated with clodronate liposome significantly improved the symptom free survival rate compared to mice treated with PBS liposome.

Figure 5.

Macrophage PPAR γ was required for the protective effect of PGZ against the development of aneurysmal rupture. Twenty mice for each group underwent the aneurysm induction. Five mice from PPAR γ flox/flox LysMCre (+/+) assigned for the vehicle treatment and five mice from PPAR γ flox/flox LysMCre (+/+) assigned for PGZ treatment were excluded because of intraoperative mortality. There were no intraoperative mortality in two PPAR γ flox/flox LysMCre (-/-) groups (both VC and PGZ group). There was no significant difference in the overall incidence of aneurysm between macrophage-specific PPAR γ knockout mice and the control mice. The activation of PPAR γ by PGZ significantly decreased the incidence of ruptured aneurysms and rupture rate in the LysMCre-negative PPAR γ flox/flox mice (control mice). However, the protective effect of PGZ was absent in LysMCre-positive PPAR γ flox/flox mice (macrophage specific PPAR γ knockout mice). A: incidence of aneurysm. B: rupture rate. C: symptom-free curve. Aneurysmal rupture in each group began to occur 7 days after the aneurysm induction.

Figure 6.

Effects of PPAR γ activation by pioglitazone (PGZ) on the infiltration of macrophages (M1/M2) and macrophage-mediated phagocytosis. (A) The number of IL12 p40 positive cell (M1) and CD68 positive cell were significantly lower in the collected tissue from PGZ-treated mice ($n = 5$) than in those from vehicle control group ($n = 6$). The ratio of M1 and M2 (M1/M2) was also lower in those from PGZ-treated mice. (B) Representative figures of immunohistochemistry compared vehicle-treated mice and PGZ-treated mice.