Subarachnoid hemorrhage from the rupture of intracranial aneurysms 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. Activation of PPAR-γ agonist, pioglitazone, 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 pioglitazone. The protective effect of pioglitazone was absent in mice lacking macrophage PPAR-γ. Pioglitazone treatment reduced the mRNA levels of inflammatory cytokines (monocyte chemoattractant factor-1, interleukin-1, and interleukin-6) that are primarily produced by macrophages in the cerebral arteries. Pioglitazone 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.

Conclusions—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. (Stroke. 2015;46:1664-1672. DOI: 10.1161/STROKEAHA.114.007722.)

Key Words: inflammation ■ intracranial aneurysm ■ subarachnoid hemorrhage
Materials 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. We detected aneurysmal rupture. 2 blinded observers performed neurological examination daily as previously described. 3 Neurological symptoms were scored as follows: 0: normal function; 1: reduced eating or drinking activity demonstrated by a weight loss >2 grams of body weight (∼10% weight loss) for 24 hours; 2: flexion of the torso and forelimbs on 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, 5–12 they were euthanized immediately (within 4 hours). Because our previous studies using this model showed that aneurysmal rupture occurs within 3 weeks of aneurysm induction, 4, 5–12 asymptomatic mice were euthanized 21 days after aneurysm induction as previously described. 5–12

The brain samples were perfused with phosphate-buffered saline (PBS), 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. Therefore, in this study, the treatments with pioglitazone (pioglitazone, PPARγ agonist, 10 mg/kg per day) 13 or both of them were started 6 days after aneurysm induction and continued until 3 weeks (21 days) after aneurysm induction. This timing of the treatment was found to affect aneurysmal rupture without affecting the formation of aneurysms. 4, 11, 12 PPARγ agonist and GW9662 were separately dissolved in 20% dimethyl sulfoxide, and then they were injected to the mice intraperitoneally once a day. Twenty percent dimethyl sulfoxide was used in the vehicle control (VC) 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 liposomes) 10, 10, 14. 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 <10% of baseline count. 15 Animals in the control group received the same volume of PBS-containing liposome (PBS liposome).

We obtained PPARγfloxed/floxed mice 16 and mice expressing Cre recombinase under the control of the myeloid-specific lysosome M (LysM) promoter from Jackson Laboratory (LysMCre17; Bar Harbor, MA). The appearance, growth, and body weight of macrophage/granulocyte-specific PPARγ knockout mice were normal. We used LysMCre-negative PPARγfloxed/floxed mice as a control and LysMCre-positive PPARγfloxed/floxed mice as macrophage/granulocyte-specific PPARγ knockout mice.

For the real-time polymerase chain reaction analysis, the total RNA was collected at 14 days after aneurysm induction from the mice that were dedicated for the mRNA analysis. Because 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 midpoint for detecting mRNA changes associated with aneurysmal rupture. We collected the control cerebral arteries, aneurysm tissues from mice that were treated with pioglitazone, and aneurysm tissues from mice that were treated with a vehicle (n=8 for each group). We assessed mRNA expression of monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α, interleukin (IL)-6, IL-1β, inducible nitric oxide synthase, and matrix metalloproteinase-9. The total RNA was extracted using the RNeasy Mini Kit (Qiagen, CA). 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). Quantitative values were obtained from the threshold cycle value (CT), and the data were analyzed by the 2−ΔΔCT method. The transcript amount of 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 a vehicle and from the mice treated with pioglitazone 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. 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) was performed to the brain tissue sections. We chose the field with the highest number of cells around middle cerebral artery for each sample and quantified the number of the infiltrated cells by cell counting (positive immunostained cells) per high-power field (×40).

Statistical Analysis

All of the results were expressed as means±SD. Fisher 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 mRNAs were compared between 2 groups using Mann–Whitney test. Statistical significance was accepted at P<0.05. GraphPad Prism 6 was used for statistics software.

Results

PPARγ Agonist Was Protective Against the Development of Intracranial Aneurysm Rupture

As a first step to investigate the roles of PPARγ in the development of intracranial aneurysmal rupture, we treated the 8- to 10-week-old male wild-type mice (C57BL/6J, The Jackson Laboratory, Maine) with a PPARγ agonist, pioglitazone, starting at 6 days after aneurysm induction (ie, 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 the vehicle and pioglitazone treatment groups (84% versus 88%; n=19 versus n=17; Figure 1A). However, compared with the vehicle treatment, the pioglitazone significantly reduced the incidence of ruptured aneurysms (Figure 1A; VC versus pioglitazone: 68% versus 29%; P<0.05) and the rupture rate (Figure 1B; VC versus pioglitazone: 81% versus 33%; P<0.05; n=16 versus n=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 pioglitazone (P<0.05). Figure 2B shows representative unruptured aneurysm, and Figure 2C shows...
ruptured aneurysm. 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 taken after the removal blood clots is shown in Figure 2C.

To further confirm the protective effect of PPARγ activation against the development of aneurysmal rupture, we added the PPARγ antagonist (GW9662) to the pioglitazone treatment group. The incidence of ruptured aneurysms and rupture rate was normalized by the treatment with GW9662 (Figure 1A and 1B; incidence of ruptured aneurysms: pioglitazone versus pioglitazone+GW9662=29% versus 62%, P<0.05, n=17 versus n=16; rupture rate: pioglitazone versus GW9662=33% versus 77%, P<0.05, n=15 versus n=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 pioglitazone-treated mice and the vehicle-treated mice (Figure I and Table I in the online-only Data Supplement). In addition, we did not observe any apparent cardiovascular complications or fluid retention.

**activation of PPARγ-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 polymerase chain reaction. Expression levels of IL-6, IL-1β, and MCP-1 were significantly lower in the aneurysm tissues collected from pioglitazone-treated mice (n=8) than in those from vehicle-treated mice (n=8; IL-6: 4.9±2.9 versus 53.8±66.2; IL-1β: 17.3±17.2 versus 37.6±20.8; MCP-1: 3.8±1.3 versus 6.2±1.7; all P<0.05; Figure 3A). As a post hoc analysis, we found that the expression levels of tumor necrosis factor-α, MCP-1, inducible nitric oxide synthase, IL-1β, and IL-6 were increased in the aneurysm tissues from vehicle-treated mice compared with control cerebral artery samples (n=8; tumor necrosis factor-α: 4.8±1.0 versus 1.0±0.5; MCP-1: 6.2±1.7 versus 1.0±0.6; inducible nitric oxide synthase: 3.5±2.5 versus 1.0±1.0; IL-1β: 37.6±20.8 versus 1.0±0.3; IL-6: 53.8±66.2 versus 1.0±1.0; all P<0.05). There was a trend that the expression level of matrix metalloproteinase-9 was lower in pioglitazone-treated mice than in vehicle-treated mice (4.24±3.5 versus 6.76±7.4; P=0.35; Figure 3A).

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

Because the activation of PPARγ by pioglitazone 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 (Figure 4A and 4B, incidence of ruptured aneurysms; PBS liposome versus clodronate liposome: 65% versus 23%, \( P < 0.05 \) and rupture rate; PBS liposome versus clodronate liposome: 76% versus 29%, \( P < 0.05 \); \( n=23 \) versus \( n=22 \)). The survival analysis also showed that mice treated with clodronate liposome significantly improved the symptom-free survival rate compared with mice treated with PBS liposome (Figure 4C; \( P < 0.05 \); \( n=19 \) versus \( n=19 \)). There was no significant difference in blood pressure between 2 groups (Figure I in the online-only Data Supplement).

**Macrophage/Granulocyte PPAR\( \gamma \) Was Required for the Protective Effect of Pioglitazone Against the Development of Aneurysmal Rupture**

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

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**Figure 3. A, Effects of peroxisome proliferator–activated receptor-\( \gamma \) (PPAR\( \gamma \)) activation by pioglitazone (PGZ) on mRNA levels of inflammatory cytokines.** Expression levels of tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)), monocyte chemoattractant factor-1 (MCP-1), inducible nitric oxide synthase (iNOS), interleukin (IL)-1 (IL-1), and IL-6 were increased in aneurysm samples compared with control cerebral artery samples. Activation of PPAR\( \gamma \) by PGZ significantly reduced the expression levels of MCP-1, IL-1, and IL-6 in aneurysm tissues. **B.** Expression of PPAR\( \gamma \) in mouse intracranial aneurysms. **Left,** In aneurysm tissues, PPAR\( \gamma \) was localized to the adventitial layer where macrophages (CD68) were abundant. **Right,** Aneurysm tissues expressed PPAR\( \gamma \) mRNA, at least, at the similar degree to the normal cerebral arteries. H&E indicates hematoxylin and eosin stain; MMP-9, matrix metalloproteinase-9; and VC, vehicle control.
There was no significant difference in the overall incidence of aneurysm between macrophage/granulocyte-specific PPARγ knockout mice and the control mice (85% versus 87%; n=20 versus n=15; Figure 5A). There was no difference in blood pressure between macrophage/granulocyte-specific PPARγ knockout mice and the control mice (Figure I in the online-only Data Supplement).

The activation of PPARγ by pioglitazone significantly decreased the incidence of ruptured aneurysms and rupture rate in the LysMCre-negative PPARγflox/flox mice (control mice). However, the protective effect of PGZ was absent in LysMCre-positive PPARγflox/flox mice (macrophage-specific PPARγ knockout mice). Aneurysmal rupture in each group began to occur 7 days after the aneurysm induction.

**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 3 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. 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. The survival analysis also showed that mice treated with clodronate liposome significantly improved the symptom-free survival rate compared with mice treated with PBS liposome.

**Figure 5.** Macrophage peroxisome proliferator–activated receptor-γ (PPARγ) was required for the protective effect of pioglitazone (PGZ) against the development of aneurysmal rupture. Twenty mice for each group underwent the aneurysm induction. Five mice from PPARγflox/flox LysMCre (+/+) assigned for the vehicle treatment and 5 mice from PARγflox/flox LysMCre (+/+) assigned for PGZ treatment were excluded because of intraoperative mortality. There were no postoperative mortality in 2 PARγflox/flox LysMCre (-/-) groups (both VC and PGZ group). There was no significant difference in the overall incidence of aneurysm between macrophage-specific PPARγ knockout mice and the control mice. The activation of PPARγ by PGZ significantly decreased the incidence of ruptured aneurysms and rupture rate in the LysMCre-negative PPARγflox/flox mice (control mice). However, the protective effect of PGZ was absent in LysMCre-positive PPARγflox/flox mice (macrophage-specific PPARγ 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.
mice) (incidence of ruptured aneurysms: VC versus pioglitazone=70% versus 30%, $P<0.05$; rupture rate: VC versus pioglitazone=82% versus 35%, $P<0.05$; Figure 5A and 5B). However, the protective effect of pioglitazone was absent in LysMCre-positive PPARγflox/flox mice (macrophage-specific PPARγ knockout mice) because there was no difference in the incidence of ruptured aneurysm or rupture rate between the LysMCre-positive PPARγflox/flox mice treated with the vehicle and the LysMCre-positive PPARγflox/flox mice treated with pioglitazone (incidence of ruptured aneurysms: VC versus pioglitazone=67% versus 60%, $P=1.0$, n=20 versus n=15; rupture rate: VC versus pioglitazone=77% versus 69%, $P=1.0$, n=17 versus 13; Figure 5A and 5B). The survival analysis using those mice that had aneurysms revealed a significant improvement of the survival by pioglitazone treatment in the LysMCre-negative PPARγflox/flox mice but not in the LysMCre-positive PPARγflox/flox mice ($P<0.05$; Figure 5C).

**Expression of PPARγ in Mouse Intracranial Aneurysms**

To confirm the expression of PPARγ in aneurysms, we assessed the expression and localization of PPARγ by the real-time reverse transcription polymerase chain reaction and

![Figure 6.](http://stroke.ahajournals.org/)

**Figure 6.** Effects of peroxisome proliferator–activated receptor–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 was 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/M2 was also lower in those from PGZ-treated mice. **B**, Representative figures of immunohistochemistry comparing vehicle-treated mice with PGZ-treated mice. HPF indicates high-power field.
Atherosclerosis is considered as a key pathological event that leads to aortic aneurysm formation, and angiotensin II–treated apolipoprotein E knockout mice. Although the development and rupture of aortic aneurysms in angiotensin II–treated apolipoprotein E knockout mice (n=6) than in those from the VC group (n=5): IL12 p40: 4±1.9 versus 0.2±0.4, P<0.01; CD68: 14.2±7.9 versus 33.2±6.4, P=0.01). The ratio of M1/M2 was also lower in the collected tissue from pioglitazone-treated mice (0.01±0.02 versus 0.16±0.1; P<0.01; Figure 6A). There was no significant difference in the number of CD36 positive cells between pioglitazone-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.

**Quantification of Macrophage Infiltration and Macrophage Phenotype**

One mouse from the pioglitazone treatment group was excluded because of intraoperative mortality. The number of IL12 p40 positive cell (M1) and CD68 positive cell was significantly lower in the collected tissue from pioglitazone-treated mice (n=5) than in those from the VC group (n=6): IL12 p40: 4±1.9 versus 0.2±0.4, P<0.01; CD68: 14.2±7.9 versus 33.2±6.4, P=0.01). The ratio of M1/M2 was also lower in the collected tissue from pioglitazone-treated mice (0.01±0.02 versus 0.16±0.1; P<0.01; Figure 6A). There was no significant difference in the number of CD36 positive cells between pioglitazone-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 PPARγ in the pathophysiology of intracranial aneurysms. Our data showed that the activation of PPARγ in macrophages and possibly granulocytes protects against the development of aneurysmal rupture. In the animal model of intracranial aneurysm, a PPARγ agonist, pioglitazone, reduced the rupture rate. The protective effect of pioglitazone seems to require macrophage/granulocyte PPARγ. Interestingly, a lack of macrophage/granulocyte PPARγ did not affect the formation of aneurysms, indicating the unique role of PPARγ 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 PPARγ may be a potential therapeutic target for the prevention of aneurysmal rupture.

Jones et al. showed the protective role of PPARγ against the development and rupture of aortic aneurysms in angiotensin II–treated apolipoprotein E knockout mice. Although both aortic aneurysm and intracranial aneurysm are morphologically similar, the underlying pathology and mechanisms are different between the 2 types of aneurysms. Atherosclerosis is considered as a key pathological event that leads to aortic aneurysm formation, and angiotensin II treatment of apolipoprotein E knockout mice causes atherosclerosis and aortic aneurysm formation simultaneously. 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. Despite different underlying pathologies among these 2 types of aneurysms, findings that activation of PPARγ 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. For example, the treatment with PPARγ agonists, including thiazolidinediones, rosiglitazone, and pioglitazone, has been proposed for aortic aneurysms. PPARγ modulates inflammation by affecting the activation of various genes. Activation of PPARγ is known to reduce the elaboration of inflammatory cytokines from monocyte/macrophages. 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 PPARγ. Previous studies that used animal models strongly suggest that excessive and sustained inflammation leads to the progression and rupture of intracranial aneurysms. Anti-inflammatory agents prevented aneurysmal rupture in mice. Clinically, the use of anti-inflammatory agent was associated with the reduced risk of aneurysmal rupture in humans. Anti-inflammatory therapy is emerging as a potential therapy for prevention of aneurysmal rupture. As a therapeutic target for modulating inflammation for the prevention of aneurysmal rupture, PPARγ may be an attractive target because it mediates expression of many inflammation-related genes and control inflammation at multiple-levels rather than affecting a single molecule or single pathway. Moreover, there are clinically available PPARγ activators, including PGZ. Although we have not fully investigated in this study, there may be additional mechanisms that are responsible for the protective effect of PPARγ activation. Such mechanisms may include the effects on matrix metalloproteinase activation, superoxide production, and expression of angiotensin II receptors.

In our study, the protective effect of PPARγ activation against the development of aneurysmal rupture required macrophage PPARγ. The similarly protective role of macrophage PPARγ was observed in the animal model of atherosclerosis. It should be noted that a lack of macrophage PPARγ 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. Although it is often assumed that there may be shared mechanisms between these 2 biological processes (ie, aneurysm formation and aneurysmal rupture), underlying mechanisms may be fundamentally different between these 2 events. Further studies are needed to elucidate the underlying mechanisms governing these processes.

Clinical studies strongly suggest roles of macrophage activation in the development of aneurysmal rupture. In contrast, CD163, a macrophage scavenger marker, was found to be significantly higher in ruptured intracranial aneurysm compared with unruptured aneurysms. 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. In our study, the infiltration of macrophages was lowered by pioglitazone treatment. In addition, the pioglitazone
treatment reduced the M1/M2 ratio. Our data suggest a causal relationship between M1 macrophages and the development of aneurysmal rupture.

There are many 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.3,10 More importantly, human intracranial aneurysms and aneurysms in this model share the common end phenotype, that is, aneurysmal rupture and associated symptoms.4,11 Future clinical studies that assess the correlation between aneurysmal rupture and the use of agents that affect PPARγ activity will be needed to validate our findings. Second, the therapy targeting rupture and the use of agents that affect PPARγ activity in the adipose tissue may affect the homeostatic regulation of metabolism needed to validate our findings. First, the animal models do not completely replicate biological events that lead to aneurysms in this model share the common end phenotype, that is, aneurysmal rupture and associated symptoms.4,11

Summary

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

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Disclosures

None.

References


Protective Role of Peroxisome Proliferator–Activated Receptor-γ in the Development of Intracranial Aneurysm Rupture

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SUPPLEMENTAL MATERIAL

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

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**Supplemental Figure:** Systolic blood pressure was measured by a tail-cuff method. There was no effect of pioglitazone (PGZ), GW9662, clodronate liposome, or PBS liposome on systolic blood pressure. There was no difference in systolic blood pressure between macrophage specific PPARγ knockout mice and the control mice. UNx: Unilateral Nephrectomy, VC: vehicle control
**Supplemental Table:** No effects of PGZ or GW9662 treatment on blood sugar level and body weight (mean ± SD).

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<tr>
<td>Body Weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle Control (VC)</td>
<td>20.1 ± 0.9</td>
<td>20.3 ± 1.1</td>
<td>20.8 ± 1.0</td>
</tr>
<tr>
<td>Pioglitazone (PGZ)</td>
<td>20.2 ± 1.4</td>
<td>20.6 ± 1.2</td>
<td>21.2 ± 1.5</td>
</tr>
<tr>
<td>PGZ &amp; GW9662</td>
<td>20.7 ± 0.9</td>
<td>20.5 ± 1.2</td>
<td>21.4 ± 1.4</td>
</tr>
<tr>
<td>GW9662</td>
<td>19.7 ± 1.1</td>
<td>21.2 ± 1.6</td>
<td>21.0 ± 1.0</td>
</tr>
<tr>
<td>PPARγ&lt;sup&gt;f/f&lt;/sup&gt;LysMcre(-)(VC)</td>
<td>19.7 ± 1.5</td>
<td>20.0 ± 1.5</td>
<td>20.4 ± 1.5</td>
</tr>
<tr>
<td>PPARγ&lt;sup&gt;f/f&lt;/sup&gt;LysMcre(-)(PGZ)</td>
<td>19.9 ± 1.8</td>
<td>19.9 ± 1.3</td>
<td>20.2 ± 1.0</td>
</tr>
<tr>
<td>PPARγ&lt;sup&gt;f/f&lt;/sup&gt;LysMcre(+)(VC)</td>
<td>19.0 ± 1.1</td>
<td>19.8 ± 0.9</td>
<td>20.7 ± 0.9</td>
</tr>
<tr>
<td>PPARγ&lt;sup&gt;f/f&lt;/sup&gt;LysMcre(+)(PGZ)</td>
<td>19.6 ± 1.2</td>
<td>20.3 ± 0.8</td>
<td>20.5 ± 1.0</td>
</tr>
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