New Mechanism of Rosiglitazone to Reduce Neointimal Hyperplasia

Activation of Glycogen Synthase Kinase-3β Followed by Inhibition of MMP-9

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Objective—Mechanism of neointimal hyperplasia after vascular injury includes activation of signaling pathways and matrix metalloproteinases (MMPs) that are involved in cell proliferation and migration. Rosiglitazone, a synthetic peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist, was reported to inhibit neointimal hyperplasia in diabetic animals and humans. But the underlying mechanism has not been clarified. In this study, we examined how rosiglitazone inhibited neointimal hyperplasia.

Methods and Results—The proliferation and survival of cultured rat VSMCs were reduced by rosiglitazone, which was mediated by inhibition of ERK and activation GSK-3β, without change of Akt. The antiproliferative effect of rosiglitazone was reversed by GSK-3β inactivation. The migration of VSMCs was also suppressed by rosiglitazone that inhibited the expression and activity MMP-9 through GSK-3β activation. Thus migration of MMP-9(−/−) VSMCs from MMP-9 knockout mice was not affected by rosiglitazone. The underlying mechanism of MMP-9 suppression by rosiglitazone was that it inhibited NF-κB DNA binding activity, which was also dependent on GSK-3β. In rat carotid artery, balloon injury significantly inactivated GSK-3β with induction of MMP-9, which was effectively prevented by rosiglitazone. Thus, rosiglitazone significantly decreased the ratio of intima to media by reducing proliferation and inducing apoptosis of VSMCs at neointima, which was reversed by inactivation of GSK-3β with adenoviral transfer of catalytically-inactive GSK-KM gene.

Conclusions—Rosiglitazone activates GSK-3β, which inhibits not only proliferation of VSMCs but also migration of VSMCs through blocking NF-κB–dependent MMP-9 activation. **(*Arterioscler Thromb Vasc Biol*. 2009;29:472-479.)**

Key Words: restenosis ■ rosiglitazone ■ GSK-3β ■ MMP-9 ■ VSMCs

After vascular injury such as balloon angioplasty, there are diverse mechanisms of VSMC proliferation and neointimal hyperplasia. There have been many studies showing that inhibition of these mechanisms can reduce neointimal hyperplasia. Among these mechanisms, the MAPK signaling pathway was reported to be activated by vascular injury and the inhibition of activated ERK pathway by drugs or gene therapy can reduce neointimal hyperplasia.[1–3] Akt/GSK pathway was also involved, as we and others showed that modulation of Akt or GSK pathway results in reduction of proliferation of VSMCs and neointimal formation.[4–6]

Rosiglitazone is a synthetic peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist, initially developed as an antidiabetic drug. Previous studies have revealed that rosiglitazone regulates gene expression of key proteins involved in lipid metabolism, vascular inflammation, and proliferation,[7–9] resulting in not only improved insulin sensitivity in patients with type 2 diabetes but also protection against development of atherosclerosis.[10,11] Recently, several studies demonstrated that rosiglitazone prevented neointimal hyperplasia after vessel injury in diabetic animals or after coronary stenting in diabetic patients.[12–15] However, the mechanism of rosiglitazone to reduce neointimal hyperplasia has not been clarified.

As a possible mechanism, we are interested in MMP-9, because PPAR-γ agonists are known to modulate the expression and activity of MMP-9 in human breast cancer cells[16,17] and macrophages.[16,17] MMP-9 was reported to play an important role in VSMCs migration and resultant neointima formation[18,19] after vessel injury. In this study, we investigated the signaling pathway by which rosiglitazone inhibits VSMC proliferation and migration both in vitro and in vivo animal model. We found that rosiglitazone prevented the

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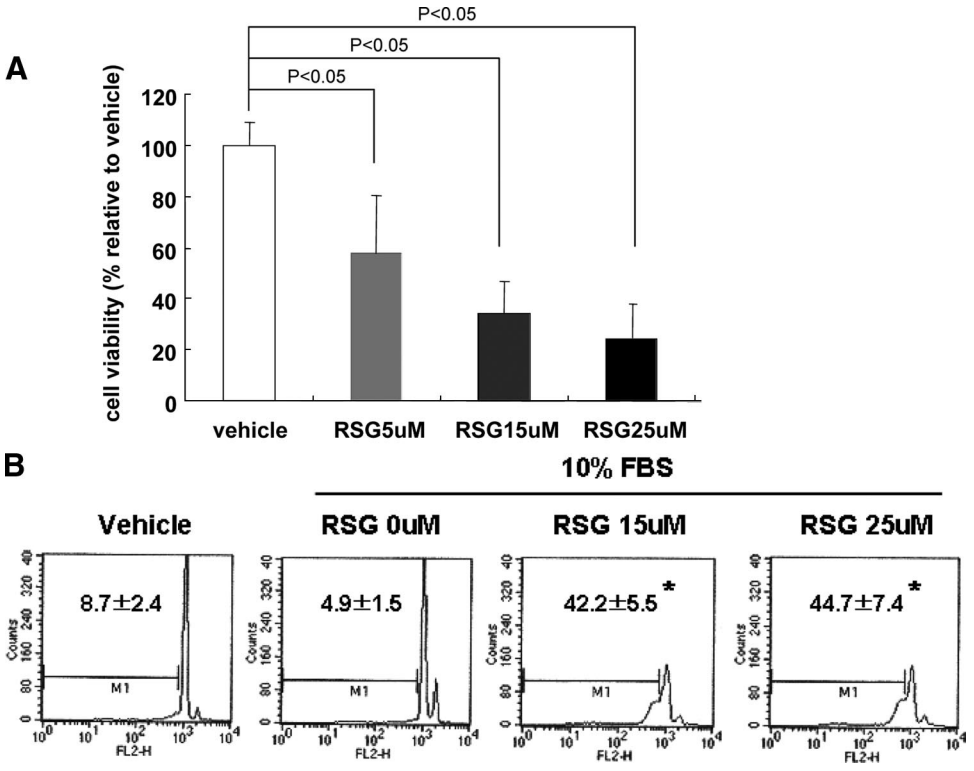

Figure 1. Rosiglitazone-induced apoptosis of VSMCs. A, WST-1 assay performed after rosiglitazone treatment to cultured VSMCs. Rosiglitazone dose-dependently reduced the viability of VSMCs. B, FACS analysis of proportion of sub-diploid cells. Rosiglitazone significantly increased apoptosis of VSMCs. All experiments were performed under FBS stimulation (*$P<0.05$ vs vehicle, n=4).

growth factor– or vascular injury–induced ERK activation/GSK-3β inactivation, without affecting Akt activity, to inhibit VSMC proliferation and migration both in vitro and in vivo. Furthermore, we confirmed that activation of GSK-3β by rosiglitazone suppressed the expression and activity of MMP-9 via blocking of NF-κB activation.

Methods

Materials and Adenoviral Vectors

Purified rosiglitazone was supplied from GlaxoSmithKline and dissolved in dimethylsulfoxide (DMSO). To evaluate GSK-3β activity, we used 2 adenoviral constructs as described previously.[4,20] Adenoviral vector expressing the nonphosphorylatable constitutively active mutant of GSK-3β with Ser switched to Ala (GSK-S9A) or the catalytically inactive GSK-3β with kinase domain mutant (GSK-KM) was used for gene delivery to modulate GSK-3β in vitro and in vivo experiments.

Cell Isolation and Culture

Rat aortic VSMCs were isolated from the thoracic aorta of Sprague-Dawley rats by enzymatic dispersion using a slight modification of a previously described method.[21] MMP-9$^{-/-}$ and their strain-matched wild-type aortic VSMCs were isolated from MMP-9 knockout or wild-type mice in the FVB/NJ background, respectively (The

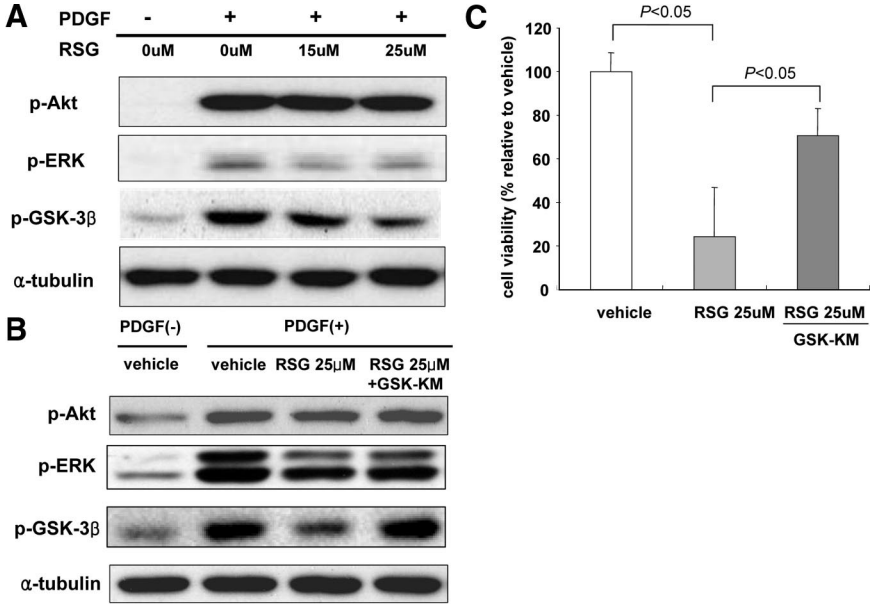

Figure 2. Inhibitory effects of rosiglitazone on VSMCs were mediated by inhibition of ERK and activation of GSK-3β but not through inhibition of Akt. A, Immunoblot analysis for phospho-Akt, phospho-ERK, and phospho-GSK-3β showed that rosiglitazone treatment resulted in a dose-dependent attenuation of ERK and GSK-3β phosphorylation, without altering the phosphorylation of Akt after PDGF stimulation. B and C, Blocking of GSK-3β with gene transfer of catalytically inactive GSK-KM significantly reversed the inhibitory effect of rosiglitazone on GSK-3β phosphorylation (B) and viability of VSMCs (C). Data are representative of at least 3 separate experiments.

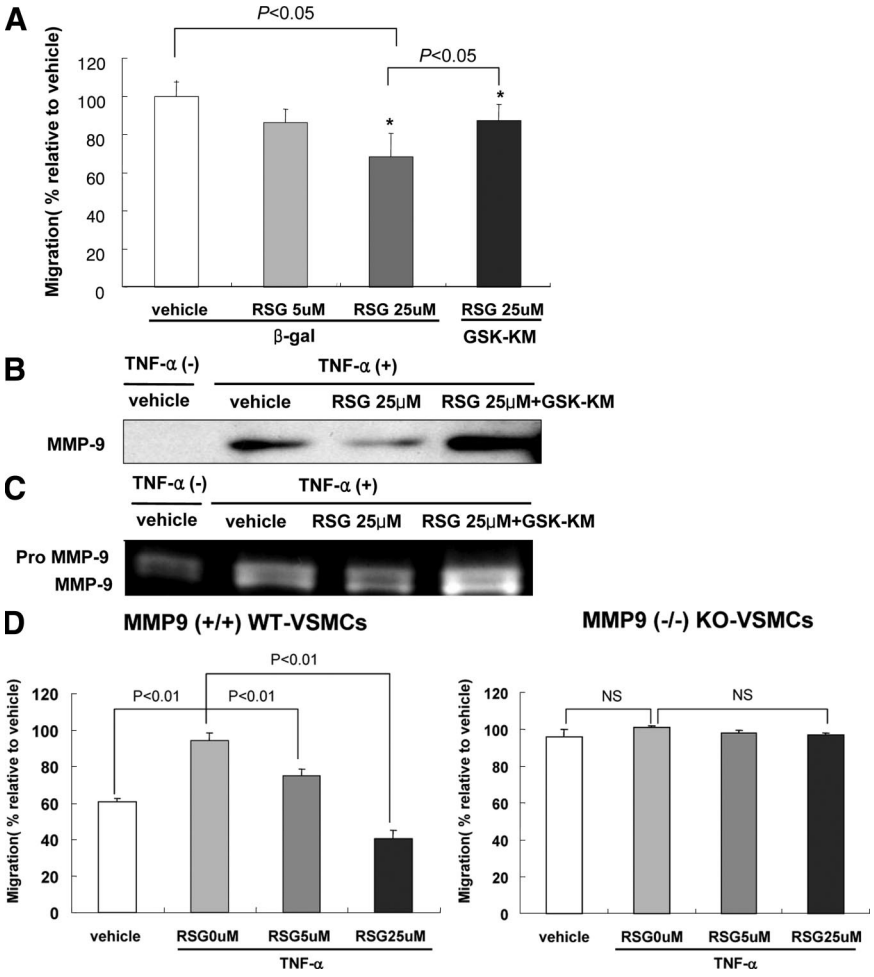

Figure 3. Rosiglitazone inhibited VSMC migration, which was mediated by MMP-9 suppression through activation of GSK-3β. A, Scratch wound assay showed the dose-dependent antimigratory effect of rosiglitazone. It was reversed by blocking of GSK-3β with catalytically inactive GSK-KM gene transfer. (*$P<0.05$, n=3). B and C, Immunoblot analysis and zymography showed that rosiglitazone reduced TNF-α–stimulated MMP-9 protein production and also enzymatic activity, which were reversed by blocking of GSK-3β with GSK-KM gene transfer. D, Rosiglitazone reduced migration of wild-type VSMCs in a dose dependent manner but not in MMP9$^{-/-}$ VSMCs. ($P<0.01$; NS indicates not significant, n=4)

Jackson Laboratory, Bar Harbor, Maine) by a slight modification of a previously described method.[21] Cells were cultured in Dulbecco Modified Eagle Media (DMEM, Gibco BRL) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 IU/mL streptomycin.

Flow Cytometry

Apoptosis were evaluated by flow cytometry, as described previously.[20] Cells were plated, allowed to attach overnight, and placed in DMEM plus 0.5% FBS for 24 hours. Rosiglitazone was then added in a serum-free medium (composed of DMEM with 10% FBS). After 24 hours, the cells were harvested and fixed in cold 90% ethanol for 20 minutes and then resuspended in staining buffer consisting of 2 mg/mL of RNase A (Roche), 20 μg/mL of propidium iodide (Sigma), and 0.1% nodient P-40. DNA content was analyzed by flow cytometry (Becton-Dickinson).

Scratch-Wound Assay

Migration of VSMCs was evaluated using the scratch-wound model as described previously.[22] VSMCs were seeded into 60-mm dishes and pretreated with rosiglitazone. After pretreatment for 1 hour, confluent monolayers were scraped through the middle of the dish by using a modified syringe needle. After wounding, monolayers were immediately washed with DMEM and incubated with 10% FBS. After 48 hours, distance from wound edge to point of cell migration was measured.

Electrophoretic Mobility Shift Assay

VSMCs were placed in DMEM plus 0.4% FBS for 24 hours. Then, VSMCs were transfected with adenoviral vectors overnight. Rosiglitazone was added and TNF-α was used for stimulation. The nuclear extract of each cell was prepared as previously described.[23] The nuclear extract was then centrifuged at 4°C for 5 minutes at 13,000 rpm, and the supernatant was either used immediately or stored at −70°C for later use. (The protein content was measured using the Bio-Rad protein assay.) The double-stranded oligonucleotides containing the consensus sequences for NF-κB (5′-CAGTGGAATTCCCCAGCC-3′) were end-labeled with [γ-^{32}P] ATP (3000Ci/mmol; Amersham Pharmacia Biotech) using T4 polynucleotide kinase (New England Biolabs) and used as probes for electrophoretic mobility shift assay (EMSA). Nuclear extract proteins (2 μg) were preincubated with the gel shift binding buffer (4% glycerol, 1 mmol/L $MgCl_2$, 0.5 mmol/L EDTA, 0.5 mmol/L dithiothreitol, 50 mmol/L NaCl, 10 mmol/L Tris-HCl(pH7.5), and 0.05 mg/mL poly (deoxyinosine-deoxycytosine) for 10 minutes, then incubated with the labeled probe for 20 minutes at room temperature. Each sample was electrophoresed in a 4% nondenaturing polyacrylamide gel in 0.5× TBE buffer at 250V for 20 minutes. The gel was dried and subjected to autoradiography.

Western Blot Analysis

Confluent cells cultured in 6-cm dishes were incubated in serum-deprived media for 48 hours. VSMCs were then stimulated using 100 ng/mL of PDGF-BB in the presence of rosiglitazone for 30 minutes. We treated VSMCs with adv-GSK-KM to block GSK-3β phosphorylation. Western blot analysis was performed as described previously.[5] The primary antibodies used were antiphospho-Akt(Ser473) (1:1000 dilution, Cell Signaling Technology), antiphospho-p44/42 MAPK (Thr202/Tyr204) (1:1000 dilution, Cell Signaling Technology), antiphospho-GSK-3β(Ser9) (1:1000 dilution, Cell Signaling Technology), anti-MMP-9 (1:400 dilution, Laboratory Vision Corp/

Neo Markers), and anti–α-tubulin (1:1000 dilution, Oncogene). The secondary antibodies used were antimouse IgG HRP (1:2500 dilution, Promega), anti-rabbit IgG HRP (1:2500 dilution, Promega). For in vivo studies, 3 carotid arteries were pooled in each group.

Immunohistochemical Staining and Fluorescence Immunocytochemistry

Immunohistochemistry (IHC) and Fluorescence Immunocytochemistry (ICC) were performed as previously described.[4,24] The primary antibodies were used anti-PC10, for proliferating cell nuclear antigen (PCNA 1:200 dilution, DAKO), anti-HA (1:200 dilution, Santa Cruz), anti-NFκB p65 (1:50 dilution, Santa Cruz). The secondary antibody was Alexa Fluor 555 goat antirabbit IgG (1:100 dilution, Molecular Probes). Sections were counterstained with methyl green or Mayer hematoxylin. All samples were coded so that analysis was performed without knowledge of which treatment each individual vessel had received.

For details methods of Cell Viability Assay, Zymography, and Rat Carotid Artery Balloon Injury Model, please see the supplement materials (available online at http://atvb.ahajournals.org).

Statistical Analysis

Data are presented as mean±SEM. Analysis was performed by ANOVA with Bonferroni test for multiple comparisons, and by the Student *t* test, using SPSS 11.0 software. A probability value of <0.05 was considered statistically significant.

Results

Suppression of Proliferation and Survival of VMSCs by Rosiglitazone via Inhibition of ERK and Activation of GSK-3β Signaling Pathways

To assess the effect of rosiglitazone on viability and proliferation of VSMCs, we performed WST-1 assay. Rosiglitazone significantly decreased the number of viable VSMCs in a dose-dependent manner (Figure 1A). In addition, we performed BrdU incorporation assay to quantify the effect of rosiglitazone on cell proliferation. As expected, we found that rosiglitazone reduced the BrdU incorporation and thus inhibited VSMCs proliferation in a dose-dependent manner (supplemental Figure I). Because reduced VSMC viability by rosiglitazone could be attributable to apoptosis, we performed flow cytometry for DNA content. The fraction of apoptotic cells significantly increased after addition of rosiglitazone as measured by flow cytometry (Figure 1B).

To examine whether these antiproliferative and proapoptotic effects of rosiglitazone were associated with a change in ERK, Akt, or GSK-3β signaling, we performed Western blot analysis. The increased phosphorylation of ERK and GSK-3β after PDGF stimulation was reduced by rosiglitazone treatment in a dose-dependent manner, whereas phosphorylation of Akt was not changed (Figure 2A). This finding was confirmed by densitometric analysis of the western blots (supplemental Figure IIA). These effects of rosiglitazone to inhibit ERK and to activate GSK-3β were reproduced by ERK inhibitor, PD098059 (supplemental Figure III). Conversely, the effects of rosiglitazone were reversed when GSK-3β was inactivated by transfection of GSK-KM which is a dominant negative form of GSK-3β, resulting in a significant increase in cell viability. It suggests that the proapoptotic effect of rosiglitazone on VSMCs is GSK-3β dependent (Figure 2B, supplemental Figure IIB and IIC).

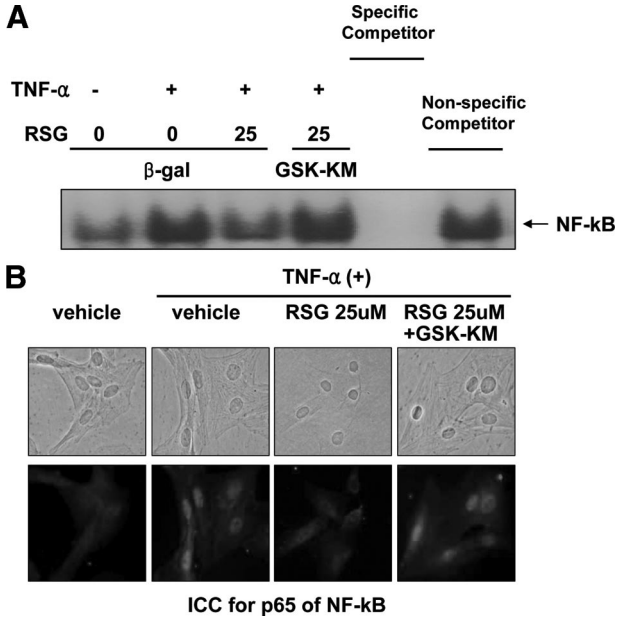

Figure 4. Rosiglitazone prevented TNF-α–induced NF-κB activation via GSK-3β. A, In EMSA, rosiglitazone decreased TNF-α–stimulated activation of NF-κB, which was reversed by blocking of GSK-3β with GSK-KM. B, By immunocytochemistry, rosiglitazone inhibited the TNF-α–induced nuclear localization of p65, which was reversed by GSK-KM.

Suppression of VSMCs Migration by Rosiglitazone via Inhibition of MMP-9 Through GSK-3β

The effects of rosiglitazone on VSMCs migration was examined using a scratch wound assay, which showed significant attenuation of VSMCs migration by rosiglitazone, and again these effects were reversed by inactivation of GSK-3β through gene transfer of GSK-KM (Figure 3A). The activity of MMP-9, a well-known modulator of VSMCs migration, was also significantly attenuated by rosiglitazone in a dose-dependent manner (supplemental Figure IV). We also confirmed that TNF-α–stimulated MMP-9 activity was decreased by rosiglitazone treatment, and that inactivation of GSK-3β completely reversed the rosiglitazone effect (Figure 3B and 3C), which was well corresponded with densitometry values of immunoblots (supplemental Figure V). Collectively, these data suggest that rosiglitazone reduced MMP-9 activity and migration of VSMCs via GSK-3β. To confirm the dependency of antimigratory effect of rosiglitazone on MMP-9 in VSMCs, we performed migration assay with MMP-9(−/−) VSMCs. As we expected, rosiglitazone inhibited the migration in wild-type VSMCs but not in MMP-9(−/−) VSMCs (Figure 3D).

Mechanism of the Suppressed MMP-9 by Rosiglitazone: the Decreased NF-κB DNA Binding Activity Through GSK-3β

To clarify the mechanism of MMP-9 inhibition by rosiglitazone, we examined the status of NF-κB activation, which is a well-known transcriptional factor for MMP-9. EMSA was performed with nuclear extracts. We found that rosiglitazone reduced TNF-α–stimulated increase in NF-κB DNA binding activity, which was reversed by inactivation of GSK-3β with

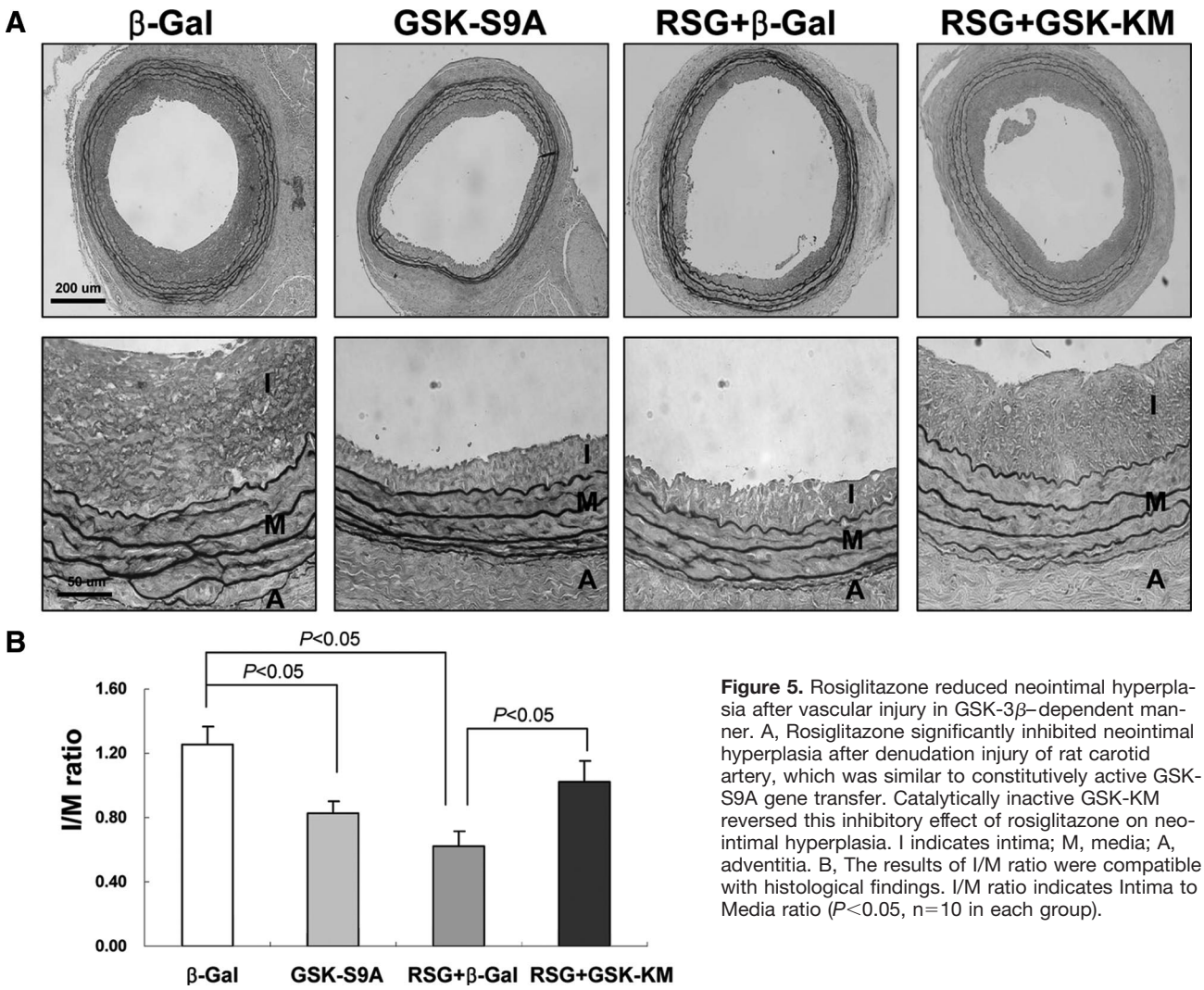

Figure 5. Rosiglitazone reduced neointimal hyperplasia after vascular injury in GSK-3β–dependent manner. A, Rosiglitazone significantly inhibited neointimal hyperplasia after denudation injury of rat carotid artery, which was similar to constitutively active GSK-S9A gene transfer. Catalytically inactive GSK-KM reversed this inhibitory effect of rosiglitazone on neointimal hyperplasia. I indicates intima; M, media; A, adventitia. B, The results of I/M ratio were compatible with histological findings. I/M ratio indicates Intima to Media ratio ($P<0.05$, n=10 in each group).

gene transfer of GSK-KM (Figure 4A). Consequently, immunocytochemistry revealed that rosiglitazone inhibited TNF-α–stimulated nuclear localization of p65, which was also reversed by GSK-KM (Figure 4B). Taken together, these results suggest that rosiglitazone reduced MMP-9 activity by suppression of NF-κB DNA binding activity, which was modulated by GSK-3β.

Reduction of Neointimal Hyperplasia by Rosiglitazone: In Vivo Evidence of GSK-3β–Mediated Actions of Rosiglitazone

From the in vitro data, we hypothesized that rosiglitazone may inhibit neointimal formation after vessel injury via activation of GSK-3β. Using a rat carotid injury model, we found that rosiglitazone significantly suppressed neointima formation at 2 weeks, which was similar to the results after gene delivery of constitutively active GSK-3β (GSK-S9A) (I/M ratio; β-gal versus GSK-S9A versus rosiglitazone+β-gal, 1.26±0.11 versus 0.83±0.07 versus 0.63±0.09 mm^2; Figure 5A and 5B). To test whether activation of GSK-3β signaling by rosiglitazone is a physiologically relevant mechanism, we blocked GSK-3β in rat carotid artery after balloon injury by delivering adenoviral vectors expressing GSK-KM genes, and found that GSK-KM gene transfer reversed the effect of rosiglitazone to inhibit intimal hyperplasia (I/M ratio; rosiglitazone+β-gal versus rosiglitazone+GSK-KM, 0.63±0.09 versus 1.03±0.13 mm^2; Figure 5A and 5B).

The effects of rosiglitazone on VSMC proliferation and apoptosis in vivo were examined using immunohistochemistry for PCNA and TUNEL assay. At 2 weeks after balloon injury, rosiglitazone significantly reduced VSMCs proliferation in neointima compared to control. Such inhibitory effect was mimicked by GSK-S9A, wheres GSK-KM abrogated the effects of rosiglitazone (% PCNA-positive; β-gal versus GSK-S9A versus rosiglitazone/β-gal versus rosiglitazone/GSK-KM, 12.3±1.2% versus 8.9±0.9% versus 8.2±0.7% versus 11.4±1.1%) (Figure 6A). In TUNEL assay, rosiglitazone significantly increased VSMC apoptosis in neointima, which was mimicked by GSK-S9A and reversed by GSK-KM (% TUNEL-positive; β-gal versus GSK-S9A versus rosiglitazone/β-gal versus rosiglitazone/GSK-KM, 6.4±1.4%, 9.2±1.1%, 8.9±0.8%, 6.2±0.8%; Figure 6B). Western blotting for phospho-GSK-3β was performed with the tissues taken before injury and 3 days after injury. The level of inactive phospho-GSK-3β at 3 days after injury was significantly elevated. Being consistent with the result of in

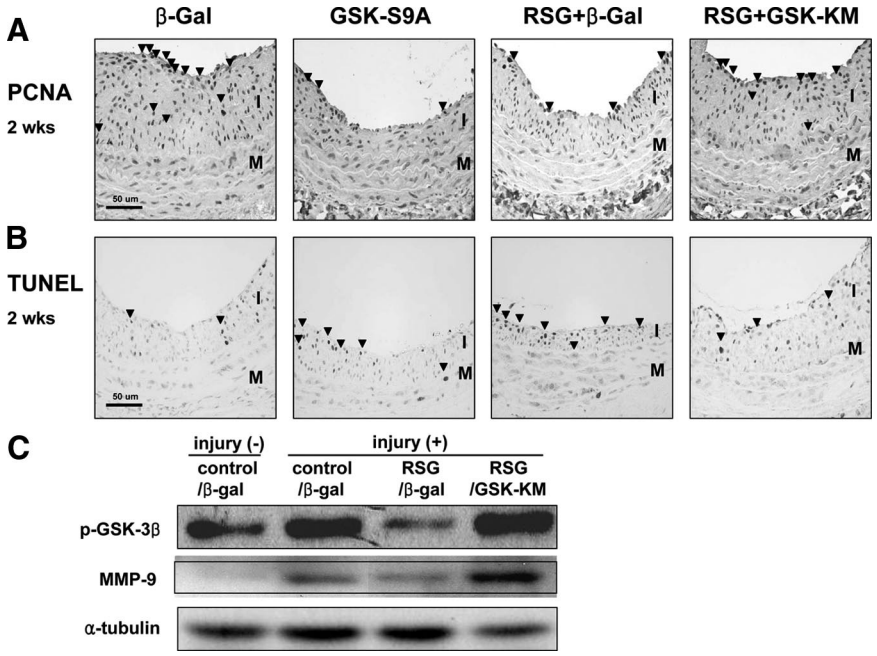

Figure 6. Effects of rosiglitazone on in vivo proliferation and apoptosis of VSMCs in neointima. A, In PCNA assay, PCNA-positive cells were significantly decreased in the rosiglitazone treatment group. Catalytically-inactive GSK-KM reversed the inhibitory effect of rosiglitazone on VSMCs proliferation. B, In TUNEL assay, TUNEL-positive cells were significantly increased in the rosiglitazone treatment group. GSK-KM reversed the effect of rosiglitazone to induce apoptosis of VSMCs in neointima. C, Western blot of arterial samples at 3 days after injury for phospho-GSK-3β and MMP-9 showed that balloon injury significantly inactivated GSK-3β presented as higher phospho-GSK-3β and induced MMP-9.

vitro experiment, the inactivation (phosphorylation) of GSK-3β after vascular injury was blocked by rosiglitazone (Figure 6C). Densitometry analysis of this immunoblot was shown in supplemental Figure VIA and VIB. The increased expression of MMP-9 after vascular injury was also significantly suppressed by treatment with rosiglitazone. This suppressive effect of rosiglitazone was in turn reversed by inhibition of GSK-3β (Figure 6C).

Discussion

Previous reports have shown that PPAR-γ agonists could prevent restenosis and atherosclerosis.[10–15] However, these studies did not show the molecular and cellular mechanisms behind the actions of rosiglitazone, especially regarding signaling mechanisms such as Akt, ERK, and GSK-3β pathways, which are well known to be activated after vascular injury such as stent implantation.[25,26] In the present study, we showed that rosiglitazone could modulate the activity of GSK-3β and ERK without altering Akt in VSMCs. Because GSK-3β is located at the nodal point where multiple cell signals merge to control the proliferation and apoptosis of VSMCs, it may represent a pharmacological target to treat restenosis after balloon injury. We showed previously that the activation of GSK-3β by gene transfer of GSK-S9A (constitutively active mutant of GSK-3β) or celecoxib, which inhibited Akt leading to activation of GSK-3β, reduces neointimal hyperplasia.[4,5] In our study, rosiglitazone treatment resulted in a significant attenuation of the inactive and phosphorylated GSK-3β and thus consequently reduced VSMCs viability and migration. Furthermore, we showed that constitutively-active GSK-S9A gene transfer results in similar findings to rosiglitazone treatment, and that catalytically inactive GSK-KM gene transfer reverses the favorable effects observed with rosiglitazone treatment, suggesting that the GSK-3β may be the key signaling molecule behind the beneficial effects of rosiglitazone on reduction of neointima formation.

Another key finding of the present study was the regulation of MMP-9 by rosiglitazone via GSK-3β. In agreement with previous studies,[16,17] we observed that rosiglitazone reduced TNF-α–induced MMP-9 expression and VSMC migration. Because previous studies have reported that TNF-α–induced MMP-9 expression is regulated by activation of the transcription factor NF-κB and AP-1,[27–29] we studied whether rosiglitazone reduces MMP-9 expression by suppressing the activation of NF-κB or AP-1. Using both EMSA and immunocytochemistry, first we found that rosiglitazone reduced the NF-κB DNA binding activity which was stimulated by TNF-α, and that this was reversed by gene transfer of GSK-KM. By using of a rat carotid injury model, we confirmed that rosiglitazone reduced the activity of NFκB in vivo via GSK-3β (supplemental Figure VII). Collectively, these data suggest that the effects of rosiglitazone on NF-κB and eventually MMP-9 are mediated by GSK-3β. Second, we also investigated whether rosiglitazone depends on AP-1 in suppressing MMP-9, but compared with NF-κB, it did not show strong effect on AP-1 DNA binding activity and translocation of c-Jun, (supplemental Figure VIII). When we stimulated the VSMCs with PDGF, much more nuclear translocation of c-Jun was observed than TNF-α stimulation. However, rosiglitazone did not completely inhibit the PDGF-induced nuclear translocation (supplemental Figure VIIIC). From our results, nuclear translocation of the NF-κB was GSK3β-dependent whereas that of c-Jun was less dependent (supplemental Figure X). The main mechanism of NF-κB nuclear translocation is the dissociation between NFκB and its inhibitory protein IκB, which can be phosphorylated by IκB kinase on serines 32 and 36 and degraded by proteolysis.[30,31] Thus we guess that GSK3β may inactivate IκB kinase and inhibit NF-κB activity. The relationship between NFκB and GSK3β requires further investigation.

It has been reported that PPAR-γ agonists could restore the impairment of the PI3K/Akt pathway that exist in insulin resistant type 2 diabetes by enhancing Akt.[29] Our results are slightly different from this report because rosiglitazone modulated GSK-3β without altering Akt activity. The reason for such a minor difference may be the different pathophysiologic states between two studies. In the previous study, the condition examined was that of diabetic patients, where impairment of Akt signaling is profound, whereas the condition in this study was vascular injury, where Akt signaling is highly activated. After vascular injury, it is well known that the ERK pathway, which is upstream regulator of GSK-3β, is also activated leading to neointima formation.[2]

In addition, it was reported that synthetic PPAR-γ agonists possess both PPAR-γ–dependent and –independent effects.[32] We found that the PPAR-γ wild-type gene transfer showed no additive effects on rosiglitazone and that PPAR-γ dominant negative gene delivery did not completely block the effects of the rosiglitazone on ERK/GSK-3β pathway (supplemental Figure IXA). Consequently, PPAR-γ antagonist, GW 9662, could not reverse the proapoptotic and antiproliferative effect of rosiglitazone (supplemental Figure IXB and IXC). Therefore, we believe that the effect of rosiglitazone on neointima reduction cannot be solely attributed to PPAR-γ itself. Although rosiglitazone may have PPAR-γ–dependent effects which may hinder neointimal growth, the activation of GSK-3β by rosiglitazone seems to be PPAR-γ independent. However, the precise interaction of synthetic PPAR-γ agonists and ERK/GSK-3β phosphorylation remains to be fully defined. Also, it is unclear whether other synthetic PPAR-γ agonists, such as pioglitazone or troglitazone, may affect the ERK/GSK3β pathway.[33,34] We believe that further studies are warranted to clarify these relationships.

In summary, we found that rosiglitazone activates GSK-3β without altering Akt, to inhibit proliferation, increase apoptosis, and attenuate migration of VSMCs. Moreover, GSK-3β activation by rosiglitazone suppresses NF-κB rather than AP-1 activity, resulting in reduced MMP-9 expression and VSMCs migration (supplemental Figure X). These effects of rosiglitazone are mainly mediated via a PPAR-γ–independent pathway. Furthermore, these in vitro effects of rosiglitazone were confirmed in vivo to reduce neointimal hyperplasia after vascular injury. Our results offer mechanistic insight into the possible role of rosiglitazone as a new therapeutic approach for proliferative vascular disease such as restenosis and atherosclerosis.

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Disclosures

None.

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