Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

Peroxisome Proliferator–Activated Receptor β/δ Alleviates Early Brain Injury After Subarachnoid Hemorrhage in Rats

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Stroke. January, 2016 (IF 5.7 (2014))

Thomas Haider

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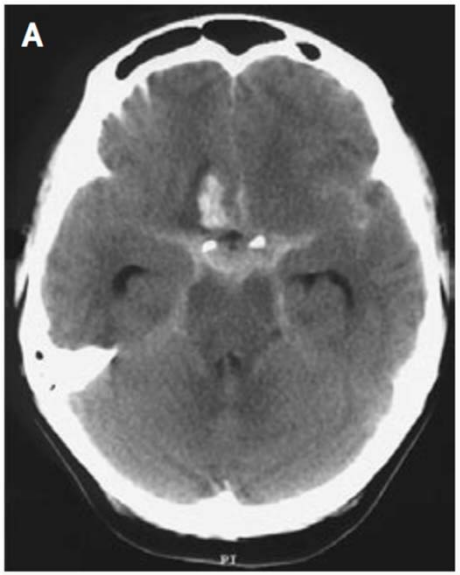
Background

Subarachnoid Hemorrhage

- Rupture of cerebral aneurysms / traumatic



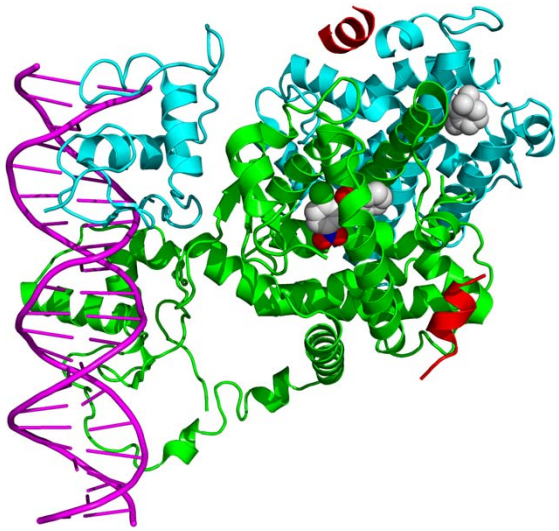
- High mortality and morbidity
  - 50% die within the first 48 hours
  - 50% of survivors – permanent disability

- Early brain injury – causative of poor outcome rather than secondary damage due to vasospasm(?)
  - Increase of ICB
  - Neuronal cell death
  - **Apoptosis**
  - Other mechanisms

Prog Neurobiol. 2012;97:14–37.
Stroke. 2016;47:196-205.
N Engl J Med 2006;354:387-96.

Peroxisome proliferator–activated receptor

- Nuclear hormone receptor
- Steroid receptor superfamily
- Co-Receptor RxR (retinoid x receptor)
- Nuclear receptor coactivator 2 - NCoA-2
- PPRE – PPAR responsive element



Prog Neurobiol. 2012;97:14–37.
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Peroxisome proliferator–activated receptor

- Subtypes
  - PPARα (Fibrates), PPARγ (Glitazone), and **PPARβ/δ**
- Energy homeostasis

- PPARβ/δ
  - skeletal muscle, adipocytes, macrophages, lungs, **brain**, and skin
  - Metabolism of fatty acids
  - Suppression of macrophage activation
  - Therapy not available yet
  - GW501516

J Adv Pharm Technol Res. 2011 Oct-Dec; 2(4): 236–240.
Wikipedia.org

Peroxisome proliferator–activated receptor β/δ

- Linked to anti-apoptotic pathogenesis of diabetes mellitus, obesity, Alzheimer's disease, spinal cord injury, Parkinson´s disease and cerebral infarction

- PPARβ/δ knockout mice -> smaller infarct size after stroke

- Role in SAH unknown

- Nuclear factor-κB (NF-κB) / matrix metallo- proteinase-9 (MMP-9) as potential downstream effectors

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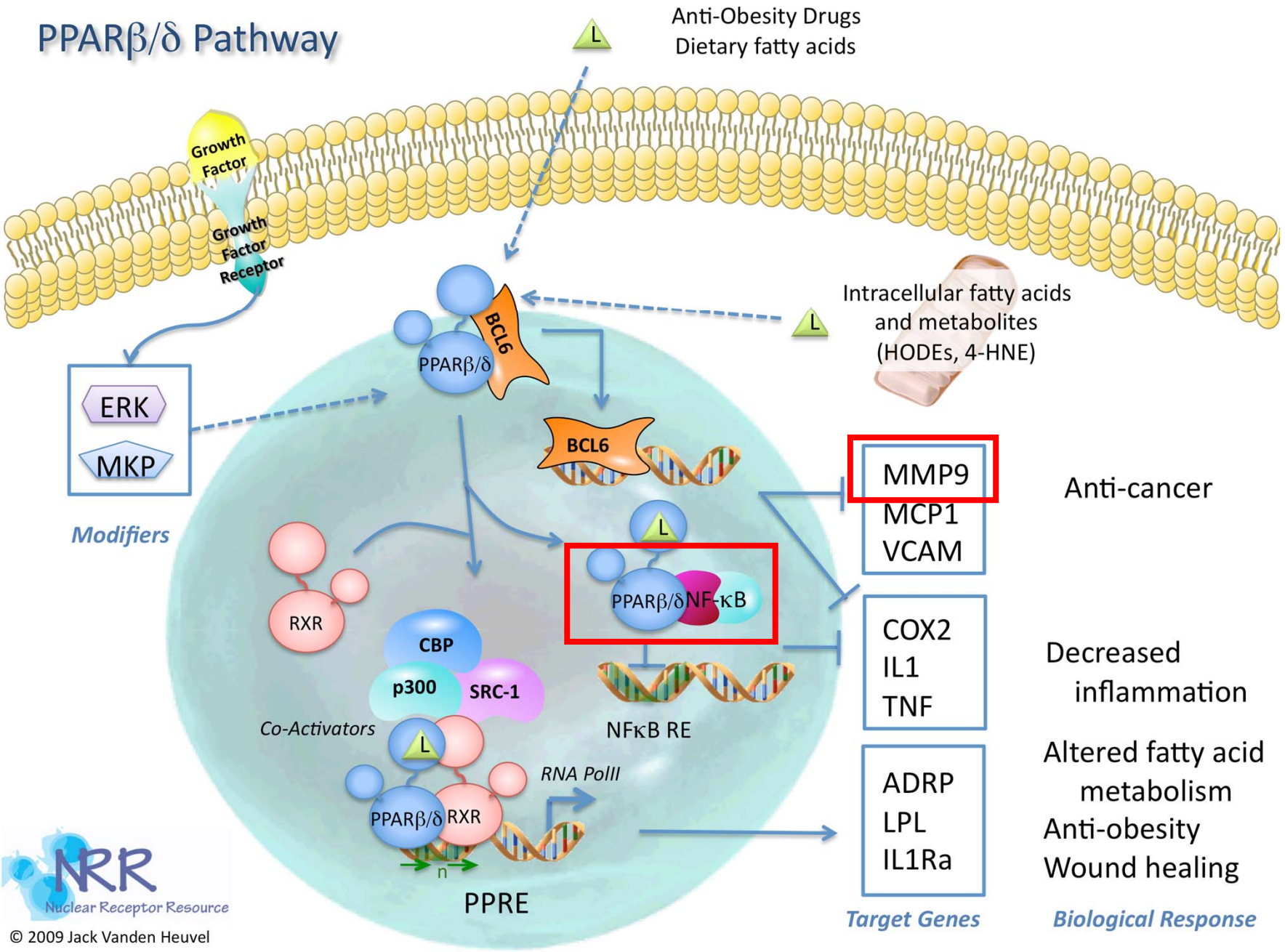
PPARβ/δ Pathway



Aim of the Study

- The aim was to elucidate…
  - …possible modulation of PPARβ/δ on NF-κB/MMP-9 pathway in the brain
  - …the potential role of PPARβ/δ and apoptosis in EBI after SAH

Stroke. 2016;47:196-205.

Materials and Methods

Experimental subarachnoid hemorrhage

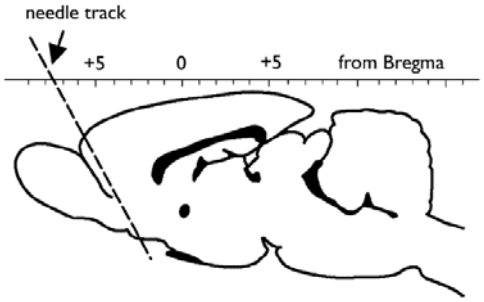


Fig. I. Schematic diagram showing the trajectory of the needle while passes between the hemispheres.

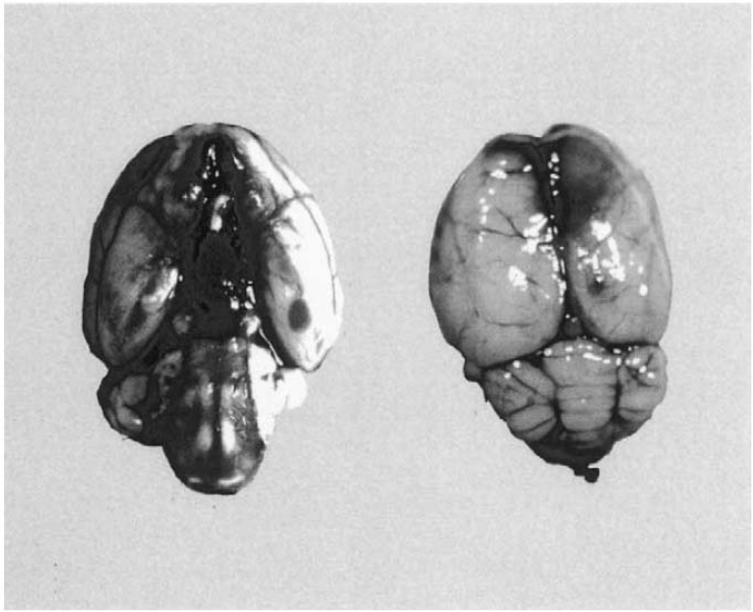


Fig. 4. Photographs showing examples of the gross appearance of the rat's brain after injection of 200 µl blood into the prechiasmatic cistern.

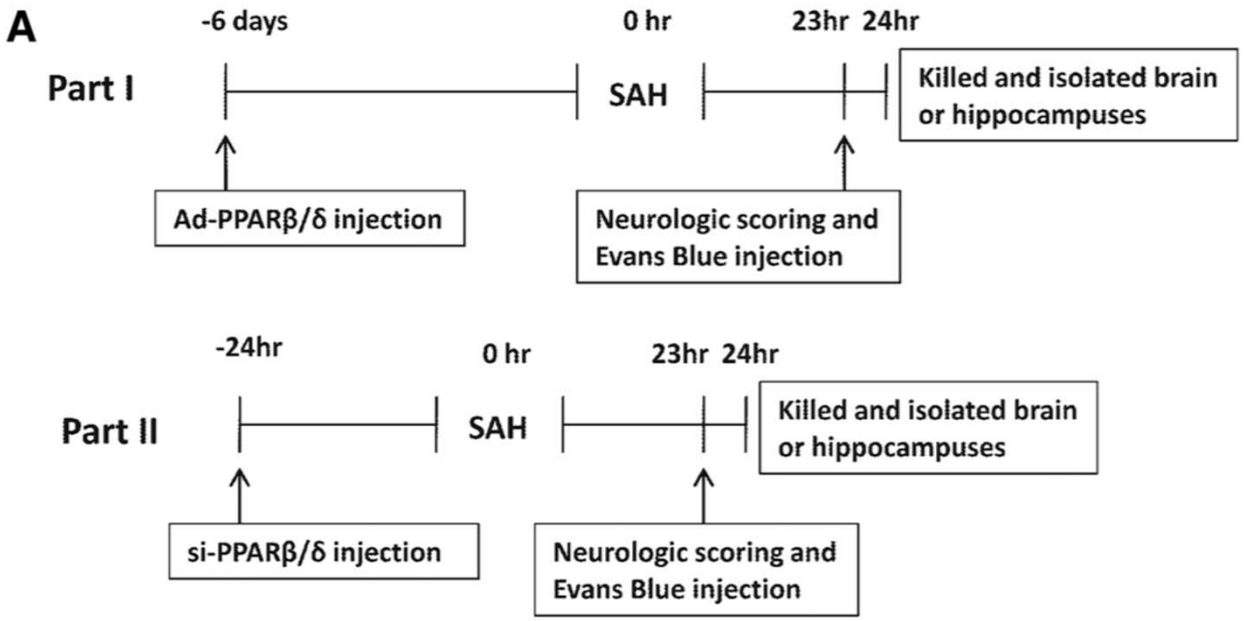
http://www.kopfinstruments.com
Prunell et al., NeuroReport, 2002

Materials and Methods

- Adenoviruses and Small Interfering RNAs
- Intracerebroventricular Injection
- Neurological Scoring (Garcia's method)
- Blood–Brain Barrier Permeability
- Brain Water Content
- TUNEL Assay
- MMP Zymography
- Immunohistochemistry
- Western Blot
- GW0742, A Special Agonist of PPARβ/δ

Stroke. 2016;47:196-205.

Timeline



Stroke. 2016;47:196-205.

Results

Mortality, BBB, Neurology at 24h

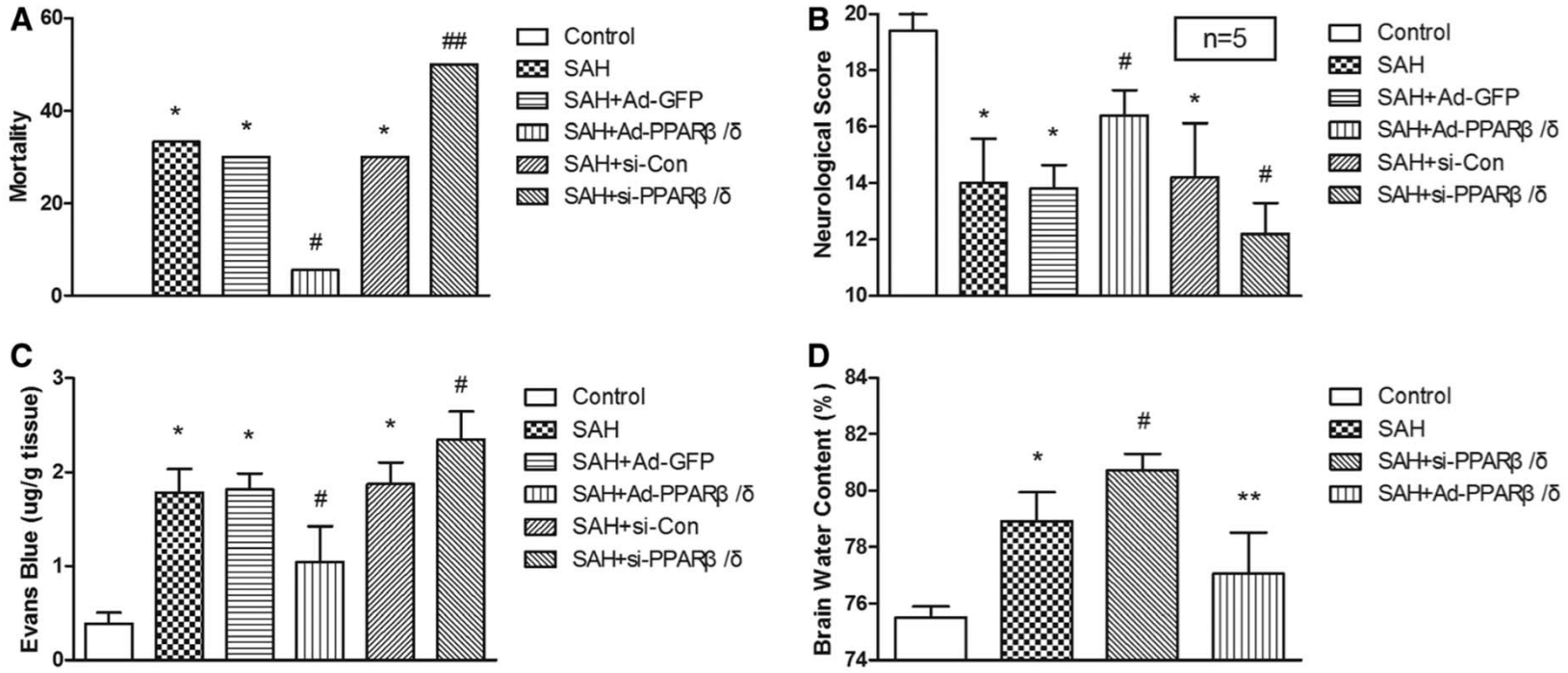


Figure 2. A, Mortality at 24 hours after subarachnoid hemorrhage (SAH), as well as adenovirus GFP (Ad-GFP) and si-CON groups, was increased obviously (**P*<0.01 vs control group); adenovirus peroxisome proliferator–activated receptor β/δ (Ad-PPARβ/δ) reduced it clearly (#*P*<0.05 vs SAH group); there was an aggravated trend after si-PPARβ/δ treatment, although no statistical difference was found between SAH group and SAH+si-PPARβ/δ group (##*P*>0.05 vs SAH group). **B**, All rats developed neurological deficits after SAH and overexpression of PPARβ/δ improved it (**P*<0.001 vs control group; #*P*<0.05 vs SAH group). **C**, Blood–brain barrier (BBB) extravasations took place in 24 hours after SAH (**P*<0.001 vs control group); the expression of PPARβ/δ prevent BBB rupture, whereas silencing PPARβ/δ promoted its injury (#*P*<0.05 vs SAH). **D**, Brain water content increased after SAH (**P*<0.01 vs control group) and enhanced more after PPARβ/δ interfering (#*P*<0.05 vs SAH). Although the overexpression of PPARβ/δ prevented brain edema after SAH (***P*=0.08 vs control group, *P*<0.05 vs SAH group).

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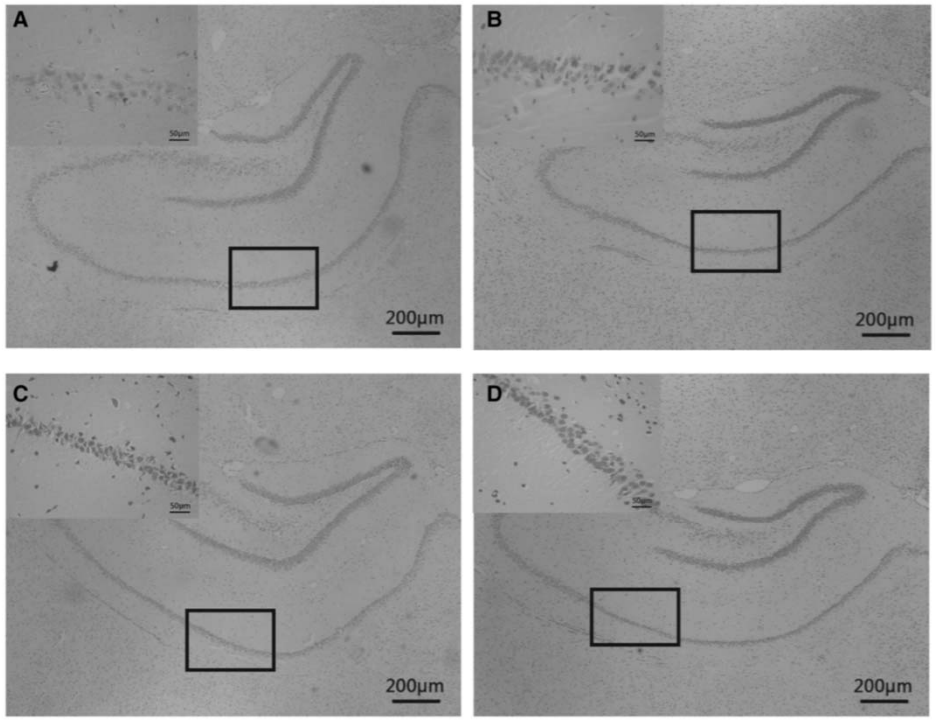
Apoptosis and PPARβ/δ in SAH



TUNEL positive cells in CA1 region of hippocampus

(x-±s, n=4)

Group	Positive cells
Control	2.25 ± 1.89
SAH	26.5 ± 6.19
SAH + Ad-PPARβ/δ	14.0 ± 2.45
SAH + si-PPARβ/δ	37.5 ± 6.56

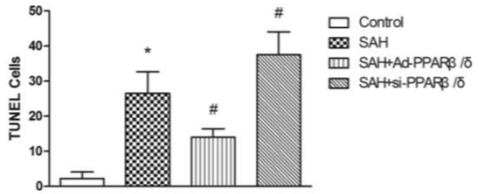


Figure 3. A, Few positive cell was observed in control group. **B**, Subarachnoid hemorrhage (SAH) operation induced considerable apoptosis of hippocampus pyramidal cells in rats. **C**, There was marked reduction of TUNEL cells by adenovirus peroxisome proliferator–activated receptor β/δ (Ad-PPARβ/δ) treatment. **D**, Neuronal apoptosis was increasing in si-PPARβ/δ group (*P<0.001 vs control group; #P<0.01 vs SAH group).

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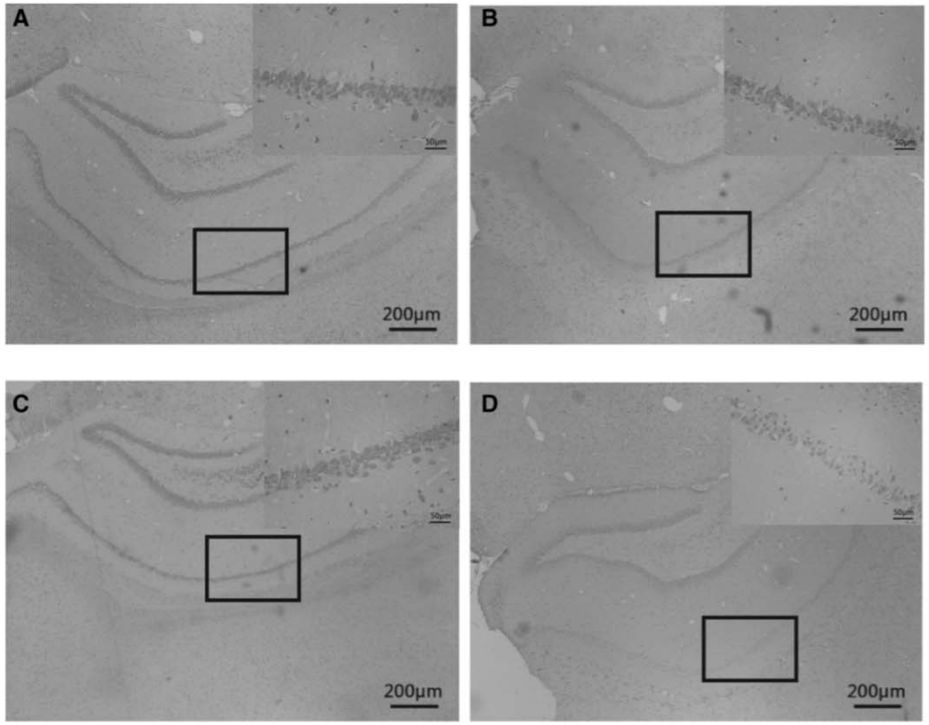
PPARβ/δ expression in SAH



PPARß/d positive cells in CA1 region of hippocampus

(x-±s, n=4)

Group	Positive cells
Control	42.6 ± 3.36
SAH	31.6 ± 7.89
SAH + Ad-PPARß/d	46.8 ± 3.35
SAH + si-PPARß/d	20.4 ± 5.37

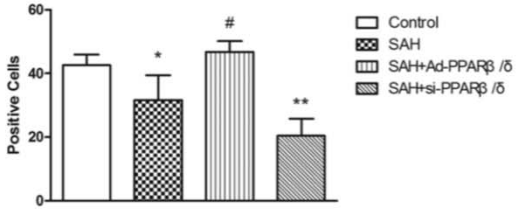


Figure 4. A, Immunohistological staining of peroxisome proliferator–activated receptor β/δ (PPARβ/δ) were seated in cytoplasm mainly in control group at 400× magnifications. **B**, PPARβ/δ-positive cells were reduced in subarachnoid hemorrhage (SAH) group (**P*<0.01 vs control group). **C** and **D**, The immunoreactivity of PPARβ/δ after Ad-PPARβ/δ and si-PPARβ/δ treatment (#*P*<0.001 and ***P*<0.01 vs SAH group).

Stroke. 2016;47:196-205.

PPARβ/δ and MMP-9 in SAH

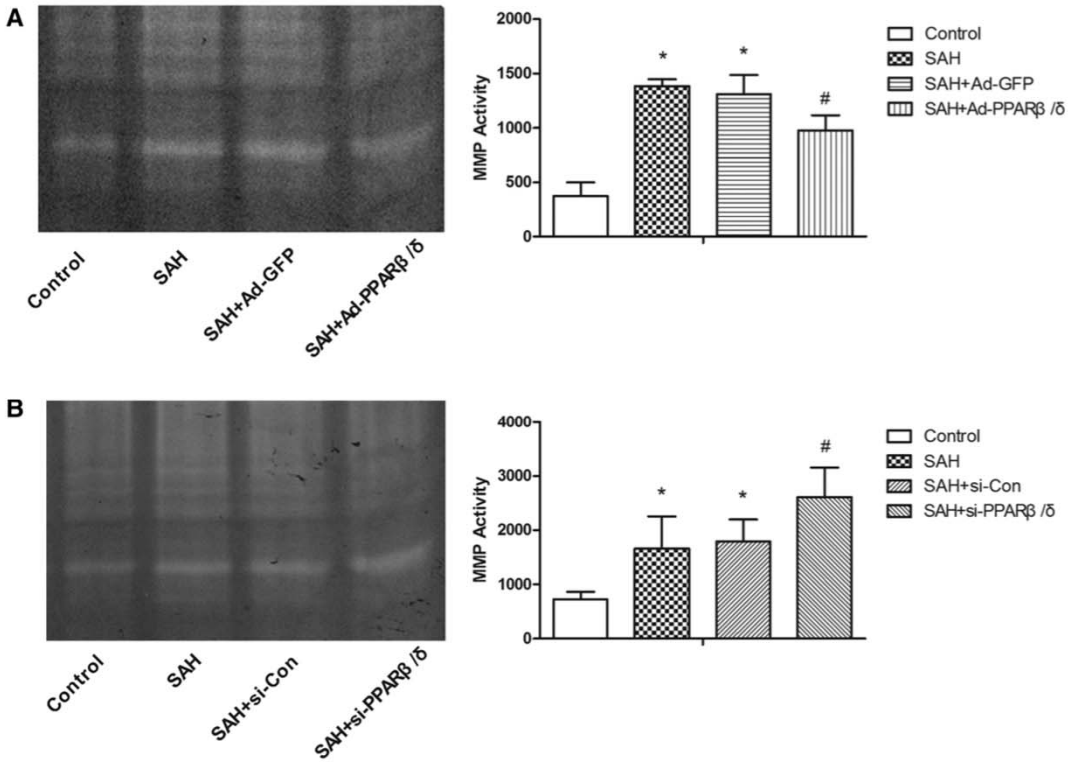


Figure 5. The region with gelatinase activity showed marked expression of active matrix metalloproteinase-9 (MMP-9; 92 kDa) in hippocampuses at 24 hours after subarachnoid hemorrhage (SAH). **A**, Activated MMP-9 was showed by densitometric analysis of highlight bands in Part I experiment (*P<0.001 vs control group; #P<0.01 vs SAH group). **B**, The variation of MMP-9 activity in Part II experiment was showed (*P<0.05 vs control group; #P<0.05 vs SAH group).

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The Role of NF-κB

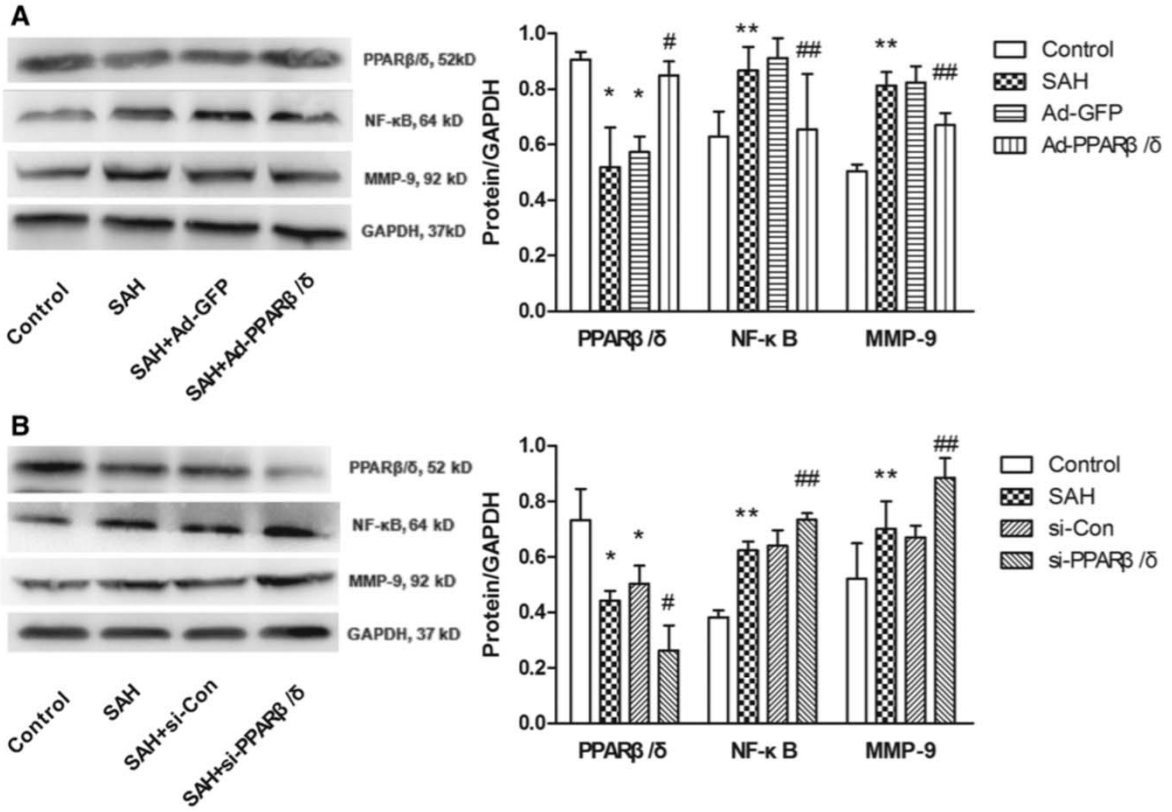

Figure 6. A, Peroxisome proliferator–activated receptor β/δ (PPARβ/δ) expression was decreased at 24 hours after subarachnoid hemorrhage (SAH; *P<0.01 vs control group) with the expression of nuclear factor-κB (NF-κB) and matrix metalloproteinase-9 (MMP-9) increased compared with control group (**P<0.01 for NF-κB and P<0.001 for MMP-9). Adenovirus (Ad)-PPARβ/δ transfection exactly increased the expression of PPARβ/δ (#P<0.01 vs SAH group and P>0.05 vs control group) and decreased the expression of NF-κB and MMP-9 as compared, respectively, with SAH group (##P<0.01 vs SAH group). **B**, PPARβ/δ expression was decreased more by RNA interference followed by SAH operation than SAH or SAH+si-PPARβ/δ group (#P<0.01 vs SAH group). Meanwhile, the expression of NF-κB and MMP-9 were enhanced greatly in comparison with SAH group (##P<0.01 of NF-κB and P<0.05 of MMP-9).

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Conclusion

Conclusion

- PPARβ/δ ↑ associated with improved outcome in SAH

- Mechanisms:
  - Downregulation of NF-κB, MMP-9
  - Inhibition of apoptosis
  - Decreased BBB-permeability

Discussion

Discussion

- Direct translation to clinics questionable
  - Side effects of available therapeutics
- Knockout model to confirm findings?
- Effect on inflammation?

Thank you for your attention!