

Introduction

Spontaneous rupture of intracranial aneurysms resulting in subarachnoid hemorrhage (SAH) represents 5% of all strokes and is associated with severe morbidity and mortality.

Growing evidence suggests that progesterone, a potent neurosteroid, can improve behavioral and functional recovery by reducing inflammation, oxidative damage, cerebral edema, and neuronal cell death after acute brain injury (1,2)

Hypothesis: Progesterone treatment would reduce cerebral arterial vasospasm and improve functional recovery in a mouse model of SAH.

Methods

Experimental Design: Adult C57BL/6 mice (n=51) were randomized to four groups for neurobehavioral analysis: Group-1 underwent sham surgery and treatment with vehicle (n=13). Group-2 underwent SAH induction and vehicle treatment (n=13), Group-3 and 4 received SAH and PRG (8 mg/kg; n=13 and 16 mg/kg; n=12 respectively). For histopathological analysis, animals (n=34) were randomized into four groups: Group-1 underwent sham surgery(n=8), Group-2 SAH and vehicle treatment(n=8), Group-3 and 4 SAH and PRG treatment (8 mg/kg;n=10 and 16 mg/kg;n=8 respectively). (Table 1)

	Behavioral group	Histopathological group
Treatment time	• 1h i.p • 6h, 24 h, 48h, 72h, 96h, 120h s.c	• 1h i.p • 6h, 24 h s.c.
Survival time	10 days	24 hours

Table 1. Treatment/survival times

Surgical Technique: Animals were placed prone, and the atlanto-occipital membrane was exposed with the aid of an operating microscope. 60 μ L of autologous blood drawn from the right femoral artery was injected into the cisterna magna. Sham group received only puncture to the atlanto-occipital membrane.

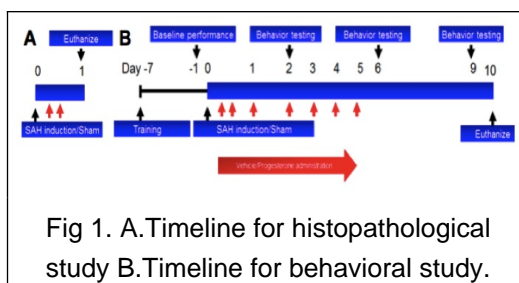


Fig 1. A. Timeline for histopathological study B. Timeline for behavioral study.

Histopathological Studies: Morphometric cerebral vasospasm was determined by measuring basilar artery perimeter in Hematoxylin-Eosin (H&E) stained cross-sections using Image-J.

Behavioral Assays: All mice were assessed using an accelerating rotarod for motor impairment. Locomotor activity was measured using the Opto-Varimex system for 5 minutes. A grip-strength meter was used to assess forelimb grip strength. The tests were carried out one day before SAH induction and 2, 6, 9 days after SAH induction. (Fig. 1)

Statistical Analysis: Histopathological study was analyzed using one-way ANOVA with post hoc LSD test. The behavioral studies were analyzed with Kruskal-Wallis test in SPSS 22.0. Results outside of mean+2SD were excluded. The criterion for statistical significance was set at $p < 0.05$.

Results

Basilar artery lumen patency was significantly increased in SAH animals treated with PRG when compared to controls ($P < 0.05$). (Table 2, Fig 2)

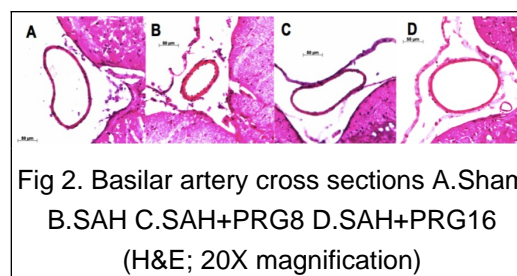


Fig 2. Basilar artery cross sections A. Sham B. SAH C. SAH+PRG 8 D. SAH+PRG 16 (H&E; 20X magnification)

SAH+vehicle-treated animals (n=11) showed decrease in motor performance as measured by latency to remain on the rotarod compared to their baseline performance, and there were significant group differences on day 6. ($p < 0.05$) (Fig. 3)

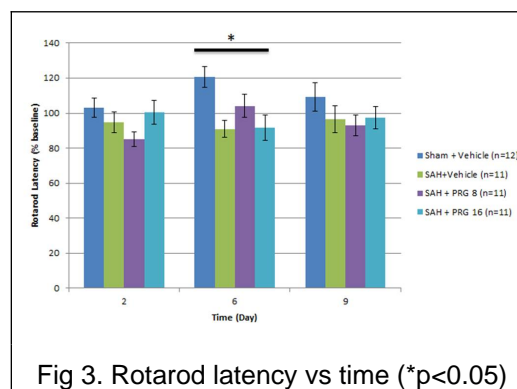


Fig 3. Rotarod latency vs time ($*p < 0.05$)

Distance travelled by SAH+vehicle-treated animals (n=12) was increased compared to Sham+vehicle-treated animals on day 6 (n=13) ($p < 0.05$). Significant group differences were found on day 6. ($p < 0.05$) (Fig. 4)

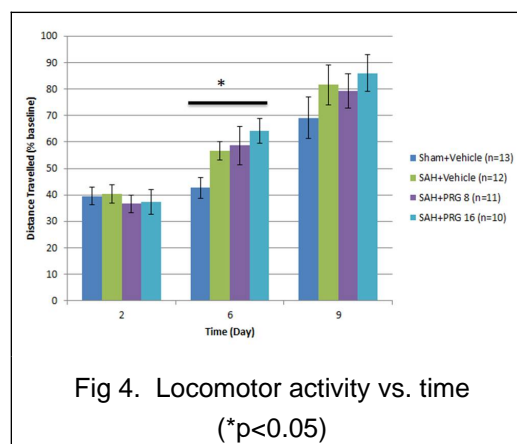


Fig 4. Locomotor activity vs. time ($*p < 0.05$)

Grip strength was measured using a grip strength meter and measures were represented as % baseline as the dependent variable (Fig. 5). Significant group differences were found on day 6 and day 9. ($p < 0.05$).

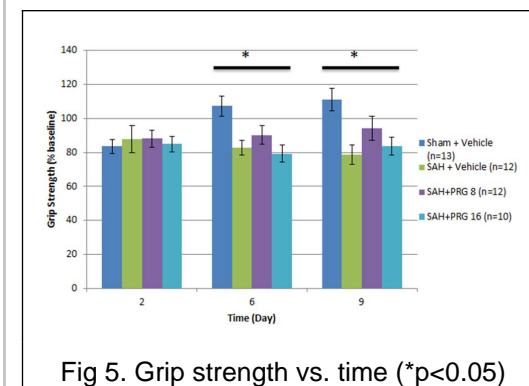


Fig 5. Grip strength vs. time ($*p < 0.05$)

Conclusion

PRG ameliorates cerebral vasospasm and may improve neurobehavioral scores in mice model of SAH. Further studies will clarify the translational potential of PRG therapy for clinical use.

References

- Wali B, Ishrat T, Won S, Stein DG, Sayeed I. Progesterone in experimental permanent stroke: A dose-response and therapeutic time-window study. *Brain*. 2014;137:486-502
- Wali B, Sayeed I, Stein DG. Improved behavioral outcomes after progesterone administration in aged male rats with traumatic brain injury. *Restor Neurol Neurosci*. 2011;29:61-71

Acknowledgements

We would like to thank Brain Aneurysm Foundation for its grant support.