Neuroprotective Effects of sAPPα Administration Following Diffuse Traumatic Brain Injury

C. Van Den Heuvel, E. Thornton, P.C. Blumbergs, K.R. Rodgers and R. Vink

Department of Pathology, University of Adelaide, Adelaide, Australia, and Centre for Neurological Diseases, Hanson Institute, Adelaide, Australia.

Summary

Soluble amyloid precursor protein α (sAPPα) is a product of the non-amyloidogenic cleavage pathway of APP that has previously been shown to have many neuroprotective functions *in vitro*. No study, however, has addressed whether sAPPα may also be neuroprotective *in vivo.* The present study has therefore examined the *in vivo* effects of sAPPα administration on axonal injury and neurological outcome following 2- metre impact/ acceleration traumatic brain injury in rats. Treatment with sAPPα at 30 min after injury (icv) significantly reduced axonal injury (AI) within the corpus callosum at 1, 3 and 7 days post-injury and significantly improved motor outcome (rotarod) compared to vehicle treated controls. Our results demonstrate the *in vivo* neuroprotective properties of post-traumatic administration of sAPPα following traumatic brain injury.

1. Introduction

Recent evidence suggests that the amyloid precursor protein (APP), a ubiquitously expressed, highly conserved integral membrane glycoprotein (Kang et al., 1987), is not only a sensitive marker of axonal injury (AI) (Blumbergs et al., 1995), but its accumulation after traumatic brain injury (TBI) may be detrimental to outcome because APP is the precursor of the neurotoxic amyloid beta (Aβ) protein found deposited within senile plaques in Alzheimer's disease (AD) (Murakami et al., 1998). In contrast, other

researchers have suggested that the increased expression of APP following TBI may, in fact, have an important reparative function following TBI. APP is upregulated acutely in injured neurones and reactive astrocytes during the early brain repair processes following TBI (Van Den Heuvel et al., 1999) raising the possibility that this increase in APP may serve a neuroprotective function (Pierce et al., 1996). A thorough appreciation of APP processing suggests that both schools of thought may be correct.

APP can be both beneficial or detrimental depending on the whether APP is post-translationally processed within cells by either of two mutually exclusive pathways. The beneficial, secreted form of APPα (sAPPα) is generated by α-secretase cleavage, while the secreted APPβ (sAPPβ) and deleterious Aβ are generated from cleavage by β and γ secretases (Hardy, 1997). Interestingly, α-secretase processing not only gives rise to sAPPα, it also precludes the formation of Aβ (Mattson, 1997) sAPPα has been reported to have many neuroprotective and neurotrophic functions within the CNS (Mattson et al., 1993). However, despite the evidence from *in vitro* studies regarding the potential benefits of sAPPα, no *in vivo* studies have examined the potential neuroprotective role of sAPPα. Therefore, one can only speculate that the increased APP expression seen following TBI may be part of a neuroprotective response to trauma. We hypothesise that increased levels of sAPPα would be protective in traumatic brain injury and accordingly, the aim of the current study is to investigate whether sAPPα administration attenuates AI and improves outcome following diffuse TBI in the rat.

2. Methods

All experimental protocols were approved and conducted according to the guidelines established for the use of animals in experimental research as outlined by the Australian National Health and Medical Research Council.

2.1 Induction of injury and sAPPα *administration*

All animals were anaesthetized using halothane and injured by the impact/acceleration model of moderate to severe diffuse traumatic brain injury as described previously (Marmarou et al., 1994). Briefly, a stainless steel disc (10 mm in diameter and 3 mm in depth) was fixed centrally onto the exposed skull between lambda and bregma and impacted by dropping a 450 g brass weight a distance of 2 m. Sham-operated controls (n=3) were surgically prepared, but were not injured. Following injury, the steel disc was removed and a 0.7mm craniotomy performed at the stereotaxic coordinates relative to the bregma: posterior 0.6 mm, lateral 1.5mm (Suehiro and Povlishock, 2001). A 30-gauge needle attached to a 25mL syringe was then stereotaxically lowered 4.0 mm then retracted 0.5 mm to facilitate icv injection into the lateral ventricle. A total of 27

animals (n=17 for histology and n=10 from outcome) received 5μL of artificial CSF (vehicle control) and a further 25 animals (n=17 for histology and n=8 for outcome) received 5μL sAPPα (0.2 mg/ml, Sigma dissolved in artificial CSF) at 30 minutes post-injury at a rate of 0.5μL per minute.

2.2 Assessment of functional motor outcome

Motor outcome was assessed using the rotarod test (Hamm et al., 1994). Briefly, the rotarod test requires an animal to walk on a motorised rotating assembly of 18 rods, each 1 mm in diameter. The rotational speed of the assembly is increased from 0 to 30 revolutions per minute (rpm) in intervals of 3 rpm every 10 s. The duration in seconds at the point at which the animal either completed the 2 min task, fell from the rods, or gripped the rods and spun for two consecutive revolutions rather than actively walking, was recorded as the task score. The rotarod data was analyzed using a one-way analysis of variance (ANOVA) followed by individual Student Newman Keuls tests. The level of significance was set at $p<0.05$.

2.3 APP immunoreactivity for axonal injury

For immunohistochemistry, animals were anaesthetised with halothane at 1 day, 3 days or 7 days after injury or sham treatment and perfused transcardially with 4% paraformaldehyde for 1 to 2 min. The brains were removed and coronally sectioned blocks (3–5-mm thick) were then processed for paraffin embedding. Serial sections (5 μm) were cut and mounted on poly-L-lysine coated slides for APP immunoreactivity. AI within the corpus callosum was semi-quantitated using a modification of our previously described grading system (Van Den Heuvel et al., 1999).

3 Results

3.1 Functional outcome

All injured animals recorded a significant decline from their pre-injury scores on day 1 following injury ($p<0.001$). In the vehicle-treated control animals, motor function remained significantly less than pre-injury values over the remainder of the 7-day assessment period. In contrast, the sAPPα treated animals improved their motor performance with repeated exposure to the task such that by day 4 after injury, their rotarod scores were no longer significantly different from their pre-injury values. At these time points, the sAPPα animals performed significantly better at the rotarod task ($p<0.05$) than the vehicle-treated control animals.

3.2 Immunocytochemistry

APP immunocytochemical analysis of AI within the corpus callosum demonstrated a reduction in the amount of AI in sAPPα treated animals compared to vehicle treated controls as early as 1-day post-injury. Profound reductions in AI were noted at both 3 and 7 days, with the reduction being most apparent at 3 days post-injury (see Figs 1A and 1B). As expected, all uninjured, sham animals showed no evidence of AI.

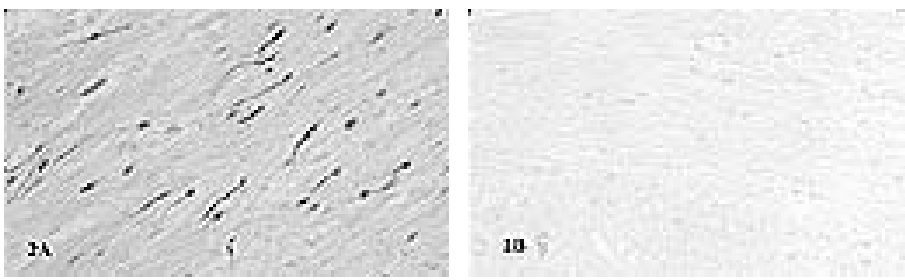

Figure 1: *Photomicrograph demonstrating AI within the corpus callosum in vehicle control animals (A) and no AI present in sAPPα treated animals (B) who survived for 3 days post-injury. (x400 original magnification).*

4. Conclusions

This is the first *in vivo* study to demonstrate the potential neuroprotective effects of sAPPα following TBI. Administration of sAPPα following moderate-severe TBI in rats significantly improves motor outcome and markedly reduces the extent of AI in injured animals.

5. References

BLUMBERGS, P. C., SCOTT, G., MANAVIS, J., WAINWRIGHT, H., SIMPSON, D. A. and MCLEAN, A. J. Topography of axonal injury as defined by amyloid precursor protein and the sector scoring method in mild and severe closed head injury. *J. Neurotrauma* 12, 565-572., 1995.

HAMM, R. J., PIKE, B. R., O'DELL, D. M., LYETH, B. G. and JENKINS, L. W. The rotarod test: An evaluation of its effectiveness in assessing motor deficits following traumatic brain injury. *J. Neurotrauma* 11, 187-196, 1994.

HARDY, J. Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci.* 20, 154-159, 1997.

KANG, J., LEMAIRE, H. G., UNTERBECK, A., et al. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* 325, 733-736, 1987.

MARMAROU, A., FODA, M. A., VAN DEN BRINK, W., CAMPBELL, J., KITA, H. and DEMETRIADOU, K. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. *J. Neurosurg.* 80, 291-300., 1994.

MATTSON, M. P. Cellular actions of b-amyloid precursor protein and it's soluble and fibrillogenic derivatives. *Physiol. Rev.* 77, 1081-1132, 1997.

MATTSON, M. P., CHENG, B., CULWELL, A. R., ESCH, F. S., LIEBERBURG, I. and RYDEL, R. E. Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein. *Neuron* 10, 243-254, 1993.

MURAKAMI, N., YAMAKI, T., IWAMOTO, Y., et al. Experimental brain injury induces expression of amyloid precursor protein, which may be related to neuronal loss in the hippocampus. *J.Neurotrauma* 15, 993-1003, 1998.

PIERCE, J. E., TROJANOWSKI, J. Q., GRAHAM, D. I., SMITH, D. H. and MCINTOSH, T. K. Immunohistochemical characterization of alterations in the distribution of amyloid precursor proteins and beta-amyloid peptide after experimental brain injury in the rat. *J Neurosci* 16, 1083-1090, 1996.

SUEHIRO, E. and POVLISHOCK, J. T. Exacerbation of traumatically induced axonal injury by rapid posthypothermic rewarming and attenuation of axonal change by cylosporin A. *J Neurosurg* 94, 493-498, 2001.

VAN DEN HEUVEL, C., BLUMBERGS, P. C., FINNIE, J. W., et al. Upregulation of amyloid precursor protein messenger RNA in response to traumatic brain injury: an ovine head impact model. *Exp. Neurol.* 159, 441-450, 1999.