p75 neurotrophin receptor (p75NTR) deficiency and pharmacological inhibition of p75NTR decrease secondary brain damage formation after experimental traumatic brain injury

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Introduction

Traumatic brain injury (TBI) initiates a complex sequence of pathophysiological changes including excessive neurotransmitter and mediator release. Neurotrophins are mediators which promote neuronal survival, differentiation, and modulate synaptic plasticity. Paradoxically, although the mature forms of neurotrophins promote neuronal survival, unprocessed forms of neurotrophins (pro-neurotrophins) induce cell death through p75 neurotrophin receptor (p75NTR) signaling. The p75NTR is widely expressed during synaptogenesis and is subsequently downregulated in the adult brain [1]. Repair-mechanisms after cerebral insults e.g. TBI can reactivate the expression of p75NTR [2]. Therefore, the question was addressed whether genetic deficiency of p75NTR or inhibition of the proapoptotic p75NTR signaling pathway might influence the outcome after experimental TBI.

Methods

Following approval of the governmental animal care committee (Koblenz, Germany) the following investigations were performed:

Model: Controlled cortical impact (CCI)
tip diameter of 3 mm, 1.5 mm brain penetration, impact duration of 150 ms, and impact velocity of 8 M/s

Anesthesia: Isoflurane (1 minute 4 vol%, followed by 2 vol%)

Animals: 127 male C57/Bl6N mice (weighing from 20.6 to 24.1 g)
p75NTR deficient animals ($NGFR^{-/-}$) and
corresponding littermates ($NGFR^{+/+}$)

Treatment: low dose (1 µM), high dose (10 µM) TAT Pep5,
or TAT peptide (TAT ctrl) 6 and 12 hours after trauma

Measurements: 1. Contusion volume in Nissl stained sections
2. Motor coordination analyzed by rotarod test
2. mRNA expression analyzed in injured cortical tissue

Statistics: Exact Wilcoxon-Mann-Whitney Test; Holm-Bonferroni Method; level of significance: P<0.05

Results

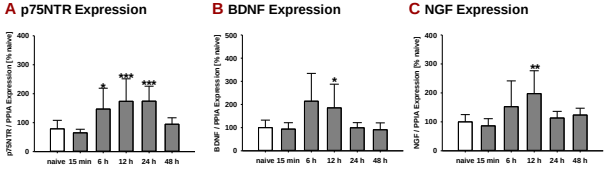

Figure 1: Influence of TBI on neurotrophin and neurotrophin receptor levels. Time course of the post-traumatic regulation of pericontusional p75NTR (A), BDNF (B), and NGF (C) expression levels following CCI was determined in naïve and after 15 min, and 6, 12, and 24 hours after trauma (1.5 mm, 8 M/s, n = 9-10 mice / group). Data are presented as mean S.D. (*) indicates P < 0.01; (**) indicates P < 0.01; (***) indicates P < 0.001.

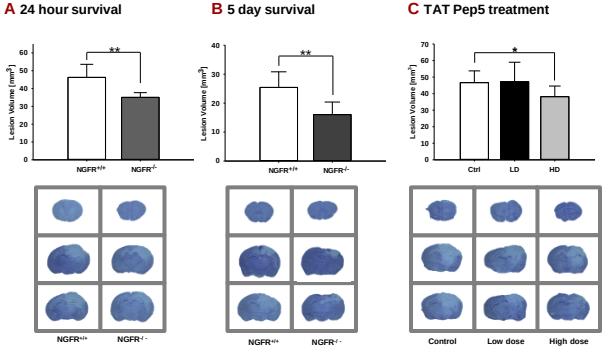

Figure 2, A,B: $NGFR^{-/-}$ mice and $NGFR^{+/+}$ littermates were randomly exposed to experimental TBI. Brain contusion volume was analyzed 24 hours and 5 days after trauma by quantification of injured brain tissue in Nissl-stained cryosections. Lesion size was significantly reduced in $NGFR^{-/-}$ compared with $NGFR^{+/+}$ at 24 hours (**A**, P = 0.004, n = 8 / group) and 5 days (**B**, P = 0.005, n = 9 / group) after trauma. **C:** Mice were randomly treated with low dose (LD) or high dose (HD) TAT Pep5, or TAT ctrl (ctrl) 6 and 12 hours after CCI. HD decreased lesion volume 24 hours after TBI(P = 0.018, n= 11 / group). Representative cresyl violet-stained sections at the coronal plane at 24 hours (**A,C**) and 5 days (**B**) after TBI. Data are expressed as mean S.D. (*) indicates P < 0.05. (**) indicates P < 0.01.

Results

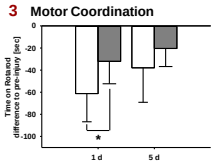

Figure 3: Effect of p75NTR deficiency on motor coordination. Motor coordination was analyzed by rotarod. The difference to baseline values in remaining on rotarod was analyzed 24 hours and 5 days after trauma in $NGFR^{-/-}$ and $NGFR^{+/+}$ mice (n = 10 mice / group). $NGFR^{-/-}$ significantly improved motor coordination compared with $NGFR^{+/+}$ at 24 hours after CCI (P = 0.014) and 5 days after injury by trend (P = 0.140). Data are expressed as mean S.D. (*) indicates P < 0.05.

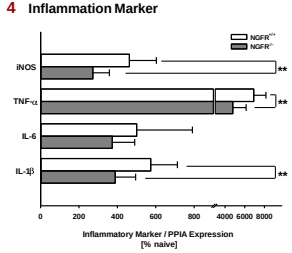

Figure 4: Effect of p75NTR deficiency on inflammatory marker genes. TBI-induced inflammatory marker gene expression levels were evaluated in peri-lesional brain tissue 5 days following brain injury. TBI increased pro-inflammatory cytokines including tumor necrotizing factor α (TNF)-α, interleukin-1 β (IL-1β), interleukin-6 (IL-6) and inducible nitric oxide synthase (iNOS). $NGFR^{-/-}$ showed markedly reduced cerebral inflammation marker expression compared with $NGFR^{+/+}$ animals (n = 10 mice / group). Data are normalized to the housekeeping gene cyclophilin A and are presented as mean S.D in percentage of naïve animals. (**) indicates P < 0.01.

Discussion

The present data suggest that p75NTR is a key activator of posttraumatic cell death, because histopathologic outcome is improved in p75NTR deficient mice. Furthermore, the p75NTR signalling inhibitor TAT Pep5 reduces brain injury after TBI. This may be of clinical interest because fusion proteins like TAT Pep5 can be intravenously administered and are delivered to the damaged brain regions [3]. Therefore, p75NTR may be a promising target to reduce neuronal cell death after cerebral insults.

References: 1. Beattie MS, 2002, Neuron 36, 375-386; 2. Shulga A, 2012, The Journal of neuroscience : the official journal of the Society for Neuroscience 32, 1757-1770; 3. Kilic, 2002, Annals of neurology 52, 617-622