Thymosin Beta4 Treatment of ICH in Spontaneously Hypertensive Rats

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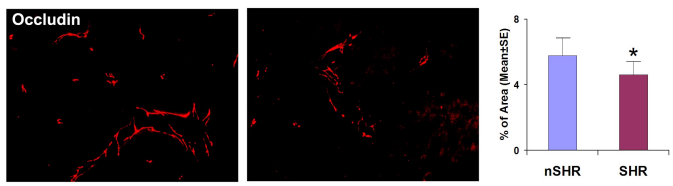
Introduction

Hypertension has been found in more than 85% of patients with intracerebral hemorrhage (ICH) and is associated with an increased mortality rate and poor neurological outcome compared with patients without hypertension. In order to bring thymosin ß4 (Tß4) closer to clinical application, we investigated the Tß4 effects on recovery after ICH in hypertensive (SHRs) and normotensive rats (nSHRs/WKY).

Methods and Results

ICH model: All experimental procedures were approved by the Institutional Animal Care and Use Committee of Henry Ford Hospital. Primary ICH was induced by direct infusion of 100 µL of fresh (nonheparinized) autologous whole blood into the right striatum adjacent to the SVZ at a constant rate of 10 µL/min. Our data demonstrated that ICH caused more severe neurological deficits in SHRs compared to nSHRs.(see below)



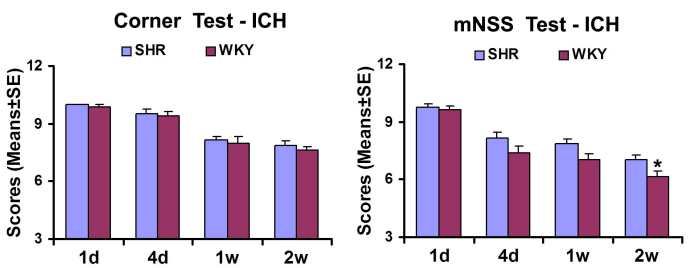
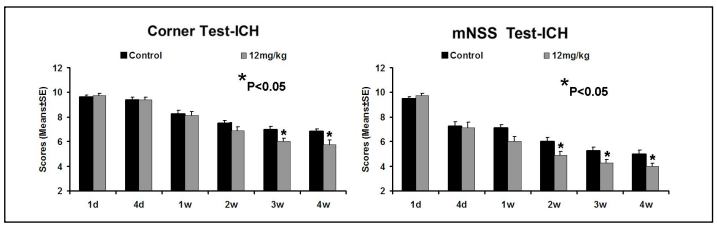


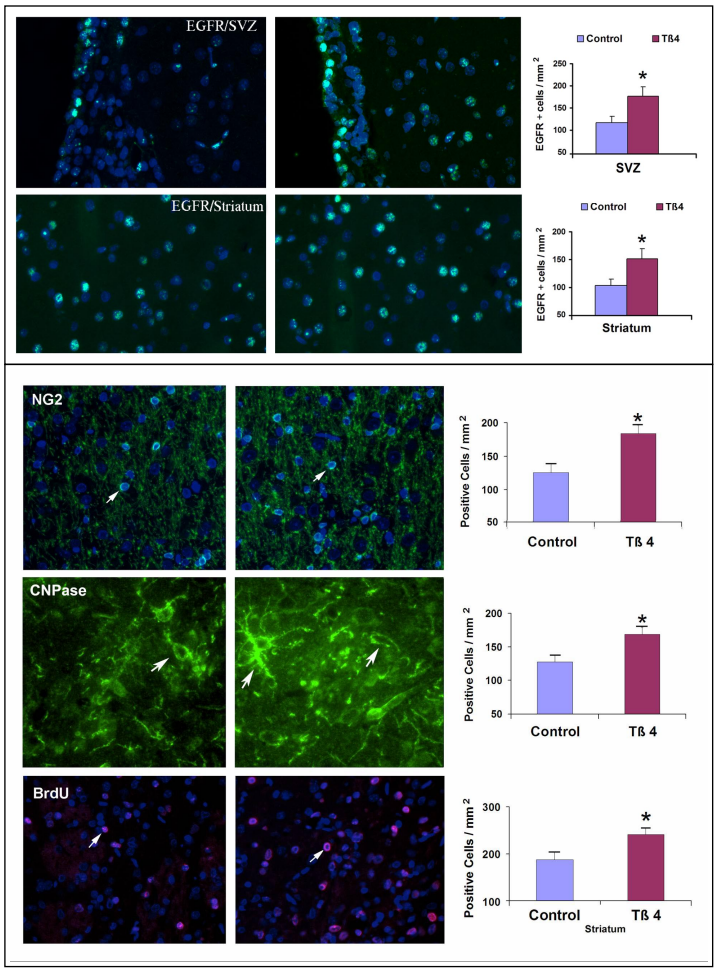
TABLE 1. Blood Presure (Mean±SD)

	Systolic Blood Pressure (mmHg)		Mean blood Pressure (mmHg)	
	SHR	WKY	SHR	WKY
Week 10	171.2± 15.6*	127.6± 10.6	144.4± 12.7*	102± 8.4
Week 11	179.1± 17.5*	129.6± 10.1	147.6± 12.2*	110± 8.6
Week 12 Intra- op	156.7± 9.4*	102± 10.3	142.5± 14.2*	84± 6.8
Week 13	172.4± 15.8*	126.7± 11.8	137.1± 16.1*	106± 7.1
Week 14	176.1± 16.4*	129.8± 12.4	147.2± 14.9*	106.9± 7.6
Week 15	181.5± 17.8*	127.6± 9.1	151.2± 14.6*	109.0± 8.1

Neurobehavioral testing: At 24 hours post-ICH, the SHR were randomly assigned into saline control (n = 8) and 12mg/kg Tß4-treated groups (n = 8). Functional outcome was assessed using a cornering test (the number of times that an animal turns to the right or left when placed in a corner) and modified neurological severity score (mNSS, a composite score of motor, sensory, balance, and reflex measures to assess neurological functions) after ICH. The result below shows that Tß4 treatment in SHR (starting at 24 hours after ICH) significantly improves corner test results and mNSS test scores at weeks 2-4 compared with the saline-treated group.



Histology and immunohistochemistry: Treated and control SHR were sacrificed 4 weeks post-ICH. Semiquantitative immunostaining measurements of BrdU (a marker for cell proliferation), 2,3-cyclic nucleotide 3'-phosphodiesterase (CNPase, a marker for maturing oligodendrocytes), chondroitin sulfate proteoglycan (NG-2, a marker of oligodendrocyte progenitor cells), and epidermal growth factor receptor (EGFR). Our results show EGFR-positive cells [as a percentage of area (with 200 µm width), in the subventricular zone (SVZ) and in the subcortical striatum in the peri-hemorrhagic striatum] were significantly increased in the Tß4 treatment SHRs compared with the saline group ($p<0.05$). NG-2 staining is increased in the striatum adjacent to the hematoma of the Tß4-treated SHRs when compared to the saline control animals. CNPase staining cells are increased in the striatum adjacent to the hematoma of the Tß4-treated SHRs compared to the saline control animals. There are significantly more BrdU cells in the Tß4 treated SHRs compared to the saline control rats.



Conclusions

- ICH caused more severe neurological deficits in SHRs compared to nSHRs.
- Tß4 treatment at 24 hours post-ICH promotes functional recovery in SHR.
- Tß4 treatment substantially amplifies cell proliferation, including proliferating OPCs and myelinating oligodendrocytes, and upregulates EGF-R expression. This was associated with substantial improvement of functional outcome after ICH.