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Combination of Vascular Endothelial and Fibroblast Growth Factor 2 for Induction of Neurogenesis and Angiogenesis after Traumatic Brain Injury

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Abstract Fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor (VEGF) are potent mitogens for endogenous neural stem cells (eNSC) and also induce angiogenesis. We infused the individual factors or their combination into the lateral ventricles of mice for 7 days after traumatic brain injury (TBI) in order to evaluate the effects on functional outcome and on eNSC proliferation and differentiation. The results show that VEGF induced a significant increment in the number of proliferating eNSC in the subventricular zone and in the perilesion cortex and that combination of FGF2 and VEGF did not augment the effects of VEGF alone. Fate analysis showed that most newborn cells differentiated into astrocytes and oligodendroglia while only a few cells differentiated into neurons. Functional outcome was significantly better in mice treated with VEGF, FGF2, or their combination as compared to vehicle. Injury size was significantly reduced only in mice treated with VEGF suggesting additional neuroprotective effects for VEGF. Combination therapy did not have an additive effect on outcome or neuronal differentiation. In conclusion, FGF2–VEGF combination does not augment neurogenesis and angiogenesis or reduce lesion volumes after TBI compared with individual factors. This may suggest the existence of a ceiling effect for brain regeneration.

Keywords Traumatic brain injury · Angiogenesis · Neurogenesis · Vascular endothelial growth factor · Fibroblast growth factor 2 · Neuroprotection

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Introduction

Neuroprotective strategies aimed at preventing cellular death after traumatic brain injury (TBI) have been largely disappointing, and to date, no neuroprotective agents are clinically available (Beauchamp et al. 2008; Leker and Shohami 2002). Repair mechanisms based on the proliferation of endogenous cells have been identified in the post brain injury, suggesting that precursors at the ventricular surface proliferate, migrate to sites of injury, and differentiate into neurons. Unfortunately, spontaneous neurogenesis is not sufficient to induce clinically significant recovery; however, augmentation of the physiologic neurogenesis may lead to improved functional outcome following injury. Thus, previous studies suggest that neurogenesis can be significantly enhanced with continuous delivery of basic fibroblast growth factor (FGF2) into the injured cortex (Leker et al. 2007) and that a combination of epidermal growth factor (EGF) and FGF2 can restore memory functions after ischemic brain injury (Nakatomi et al. 2002). However, merely augmenting the number of newborn cells in the brain may not suffice as these cells depend on newly formed blood vessels for their metabolic and trophic needs (Gage et al. 1998; Horner and Palmer 2003; Palmer et al.

2000). Vascular endothelial growth factor (VEGF) is a trophic factor that is expressed in the CNS following injury (Dore-Duffy et al. 2007; Lafuente et al. 2006; Papavassiliou et al. 1997; Skold et al. 2005; Wang et al. 2005) and induces angiogenesis (Kim et al. 2004; Krum and Rosenstein 1998; Nag et al. 1997; Nieto et al. 2001). VEGF may also have beneficial effects on the survival of newborn neuronal precursors (Widenfalk et al. 2003) and has been implicated in neurogenesis (Fabel et al. 2003; Wang et al. 2007; Xiao et al. 2007) and neurite outgrowth (Jin et al. 2006). Inhibition of VEGF expression after injury may actually exacerbate outcome (Skold et al. 2006). Indeed, we have previously shown that exogenous VEGF reduces lesion volume and induces neurogenesis and angiogenesis after traumatic brain injury (Thau-Zuchman et al. 2010). Given the effects of FGF2 and VEGF on neurogenesis and angiogenesis, the present study was designed to explore the effect of continuous infusion of a combination of FGF2 and VEGF into the lateral ventricles of mice on functional recovery and stem cells proliferation after closed head injury (CHI).

Materials and Methods

Animals and Traumatic Brain Injury Model

The study was conducted according to Institutional Animal Care and use Committee guidelines in compliance with NIH guidelines. Adult Sabra male mice weighing 40 g (n=39) were used for these experiments and treated with vehicle (n=10), VEGF (n=8), FGF2 (n=11), or the combination of FGF2+VEGF (n=10). Food and water were provided ad libitum. TBI was induced in mice using the CHI model (Chen et al. 1996; Flierl et al. 2009). This is a highly reproducible model of focal TBI that results in isolated frontoparietal cortical injury. Briefly, after induction of isoflurane anesthesia, a midline longitudinal incision was performed; the skin was retracted and the skull exposed. The left anterior frontal area was identified and a tipped Teflon cone was placed 1 mm lateral to the midline. The head was fixed and a 95-g weight was dropped on the cone from a height of 18 cm (Chen et al. 1996; Flierl et al. 2009). During surgery, the animals were implanted with an Alzet mini-osmotic pump secreting vehicle (saline) or VEGF (10 μg/ml)/FGF2 (2.5 μg/ml)/VEGF + FGF2 for 7 days at a rate of 0.5 μl/h for a total dose of 0.84 μg into the right lateral ventricle. After recovery from anesthesia, the mice were returned to their home cages with postoperative care and free access to food and water. At day 10 after CHI, all pumps were removed.

BrdU Injections

From day 4 post injury, and during 10 days, all animals received IP injection of the tracer BrdU (50 mg/kg, twice a day) to label dividing cells.

Neurobehavioral Evaluation

At 1 h after CHI, the functional status of the mice was evaluated with a set of 10 neurobehavioral tasks (neurological severity score, NSS), which examine reflexes, alertness, coordination, motor abilities, and balancing (Beni-Adani et al. 2001). The pathological scores correlate very well with clinical disability scores and with the degree of brain edema (Tsenter et al. 2008). Failure to perform a task scores 1 point and a success scores 0. Hence, normal animals score 0, reflecting healthy mice, whereas a score of 10 reflects maximal neurological impairment. The first NSS was obtained 1 h after TBI, and it reflects the initial severity of injury. NSS at 1 h is predictive of both mortality and morbidity and it also correlates well with the extent of damage seen on MRI (Tsenter et al. 2008). Therefore, we selected only animals with an NSS of 6–7 at 1 h for this test (NSS 1 h). The extent of recovery is calculated as the difference between the NSS at 1 h and at any subsequent time point (ΔNSS). Immediately after evaluation of NSS at 1 h, the mice were randomly assigned to treatment groups and NSS was reevaluated on days 1, 3, 5, 7, and 14 and in 1-week intervals up to 90 days after CHI. The analyses were performed by an investigator that was blinded to treatment.

Injury Size

At 90 days after surgery, the animals were deeply anesthetized and perfusion fixed with 4% paraformaldehyde. Brains were frozen-sectioned at 10 μm. Brain slices 200-μm apart between bregma +1.42 and bregma −0.8 were stained with Giemsa stain-modified solution (Fluka, Sigma-Aldrich Corporate, St. Louis, MO, USA; 1:1) and digitally photographed. The volume of injured tissue was measured with image J.40 g software (National Institutes of Health, USA). Damaged tissue volume was calculated by dividing the volume of the injured hemisphere by that of the non-lesioned hemisphere (Swanson et al. 1990) The results are expressed as a percentage of hemispheric tissue :

$$\frac{\text{Area of contralateral hemisphere} - \text{Area of ipsilateral hemisphere})}{\text{Area of contralateral hemispher}} \times 100 = \text{lesion volume (\%)}$$

Immunohistochemistry

Overall, 12 evenly spaced slices were counted for each brain between bregma +1.42 and bregma −0.8 and in each slice 10 evenly spaced high power magnification fields (×400) in the entire area surrounding the infarct/CC/subventricular zone (SVZ) were counted. Because by 90 days post CHI, the lesion core had already liquefied; the area forming the outer boundary of the brain represents the border zone that survived. This area included mainly cortex and subcortical tissue but not striatum. Brain slices were double or triple stained for immunohistochemical evaluation using fate-specific antibodies that included: rat anti BrdU (marker for cell proliferation; Accurate NY, NY, USA; 1:200); rabbit anti GFAP (marker for astrocytes; Dako, Glostrup, Denmark; 1:200); mouse anti NeuN (marker for mature neurons 1:100) and rabbit anti Gal-C (marker for oligodendrocytes; 1:200) all from Chemicom, Temecula, CA, USA, and mouse anti CD31 (marker for endothelial cells; Abcam Inc. Cambridge, MA, USA; 1:100). Alexa 488 and Alexa 555 conjugates were used as secondary antibodies (Molecular Probes, Leiden, The Netherlands; 1:200) and DAPI (Sigma, Israel) was used to visualize nuclei.

Cell Counting

Immunopositive cells were counted using an epifluorescent Olympus microscope in prespecified regions of interest (ROI) including the SVZ, corpus callosum, and the area surrounding the lesioned cortex. Overall, 10 equidistant fields were counted per region of interest (30 fields per slide and 360 fields per brain at ×400 magnification). Confocal Z sections on a ZEISS system were used to determine colocalization.

Statistical Analysis

Analysis was performed with the Sigma-Stat software package (Systat., Richmond, CA, USA). Data are presented as mean ± SEM. Values were compared using one-way analysis of variance followed by post test Dunnett's Multiple Comparison Method. P values ≤0.05 were considered significant for all comparisons.

Results

Treatment with VEGF, FGF2, or Their Combination Significantly Increases Functional Gain after CHI

Neurological deficits were assessed at predetermined time points after CHI using the NSS. A more rapid and pronounced improvement in NSS was observed in VEGF-treated animals, as compared with FGF2-treated mice, the combined therapy, or vehicle. The absolute NSS was lower (Fig. 1) and the ΔNSS from day 1 to 90 was significantly greater in animals treated with VEGF (data not shown). The differences in NSS in the VEGF-treated group were apparent as early as day 3 after CHI suggesting an early neuroprotective effect and the difference continued to increase with time and sustained until the conclusion of the study in all treatment groups vs. vehicle. Of note, the slope of NSS changes with time significantly increased after 14 days and the plot diverged significantly more from vehicle in all active treatment groups at this time point similar to what was seen after cerebral ischemia (Shetty et al. 2004). However, the combination of VEGF and FGF2 did not further improve outcome as compared with VEGF alone.

Treatment with VEGF, FGF2, or Their Combination Decreases Lesion Size after Injury

A 50% reduction in lesion size was identified at 90 days post CHI in all treated mice (Fig. 2), implying a neuroprotective effect for exogenous FGF2 or VEGF. However, in mice treated with the combination therapy, no additional reduction in lesion size compared with those treated with either growth factor alone was observed.

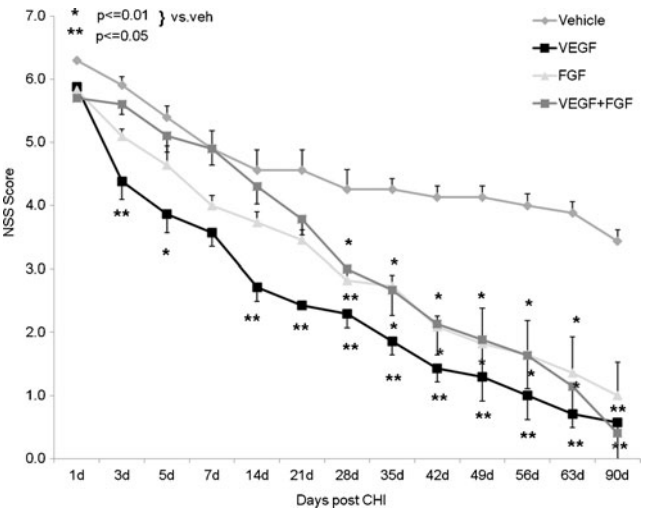

Fig. 1 VEGF, FGF2, and their combination improve neurological disability. Animals underwent CHI and were treated with VEGF, FGF2, their combination, or vehicle. Neurological disability was evaluated at different time points post CHI with the neurological severity score (NSS). Mice treated with VEGF showed significantly larger improvement beginning at day 3 post CHI and sustaining until the end of the study, 90 days post injury. Mice treated with the combination of VEGF and FGF2 did not yield further improvement compared with those treated with either factor alone

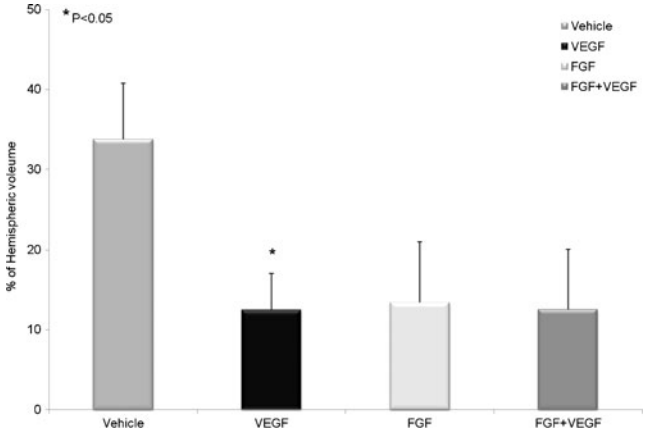

Fig. 2 VEGF, FGF2, and their combination reduce lesion size. Ninety days after injury, the volume of injured tissue was measured in 18 equidistant brain slices stained with Giemsa. All three treatments reduced lesion size compared to vehicle; however, only in VEGF-treated group that this difference reached significance

VEGF and Combined VEGF/FGF2 Therapy Increase Cell Proliferation after Injury

To assess the effect of all three treatments on cell division, the total number of newborn BrdU + cells was measured at 90 days post injury. A significant increase in the number of BrdU + cells was found in mice treated with VEGF or the combination VEGF/FGF2 at the ROI including the corpus callosum (1.6- and 1.9-fold increase, respectively) and the area surrounding the lesioned cortex (both 1.7-fold increase) (Fig. 3). In contrast, treatment with FGF2 at the dose given here did not lead to a significant increment in cell proliferation at all ROI. At 90 days post CHI, most BrdU + cells were localized to the immediate perilesion area suggesting accumulation of newborn cells around the injured brain area. These results suggest a possible mitogenic or prosurvival effect of VEGF and combined FGF/VEGF therapy on dividing cells. Of note, combination therapy did not lead to an additional increase in cellular proliferation as compared with VEGF alone.

Treatment Effects on Newborn Cell Differentiation after Injury

To determine the fates of newborn cells after CHI, we used immunohistochemical methods with double and triple labeling and confocal microscopy with Z sectioning for colocalization. Most newborn cells in all treatment groups differentiated into astrocytes (GFAP+; Fig. 4a) in the perilesioned cortex. Newborn cell differentiation into oligodendroglia (Gal-C+; Fig.4b) occurred mainly in the corpus callosum. A smaller amount of BrdU-positive cells also expressed markers for mature neurons (NeuN+; Fig. 4c) in the injured cortex, suggesting site appropriate differentiation of the newborn cells. Overall, the absolute numbers of mature neurons at the lesion border was significantly higher in VEGF treated mice and in the combined FGF/VEGF therapy vs. FGF2 (3 and 2 vs. 1.5-fold increase vs. vehicle-treated mice, respectively $p=0.005$)), whereas all active treatments significantly increased astrocytic numbers (2.8 vs. 2 or 2.4-fold increase vs. vehicle-treated mice, respectively $p<0.01$). In contrast, the number of newborn oligodendroglia that were found in the corpus callosum were higher in VEGF and in

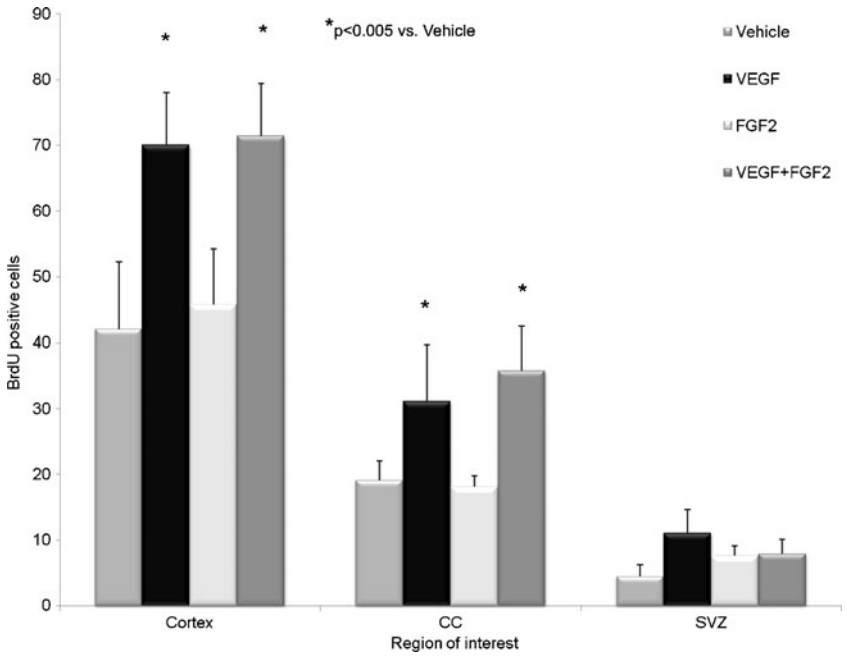

Fig. 3 VEGF, FGF2, and their combination increase cell proliferation after TBI. Animals were injected with BrdU following CHI. The absolute number of BrdU + cells was measured at 90 days post TBI at various regions of interest including the subventricular zone, the corpus callosum, and the cortex on the side of injury. Note that VEGF led to significantly more BrdU + cells in the cortex surrounding the injury

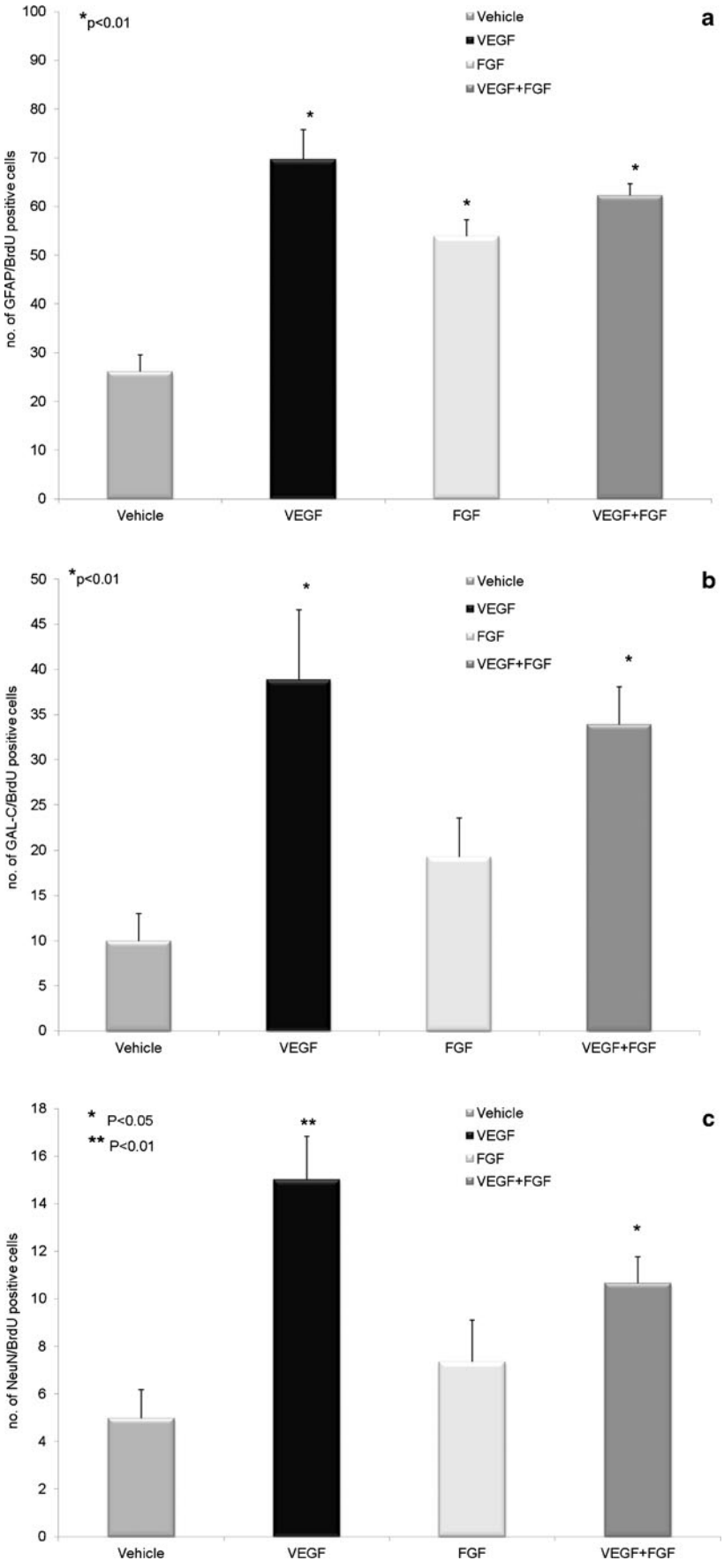

Fig. 4 Effects of VEGF, FGF2, or their combination on newborn cell differentiation. Animals underwent CHI and were killed 90 days later. Newborn cell fate was determined with immunohistochemistry. Most newborn cells in animals treated with VEGF differentiated into GFAP + astrocytes in the perilesion cortex (**a**). The number of newborn astrocytes was significantly larger in all treatment groups compared with controls. At the corpus callosum, most newborn cells differentiated into Gal-C + oligodendroglia (**b**). The number of newborn oligodendroglia was significantly larger in VEGF and VEGF + FGF treatment groups. The numbers of NeuN/BrdU double positive newborn mature neurons were significantly larger in the cortical area surrounding the lesion of VEGF and VEGF + FGF treatment groups (**c**)

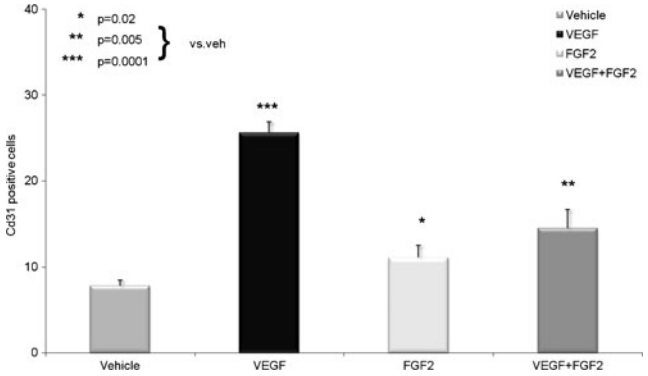

Fig. 5 VEGF, FGF2, and their combination increase angiogenesis after TBI. Animals were killed 90 days after CHI and the number of blood vessels was counted in the perilesion cortex. Significantly more vessels were seen in the cortical area surrounding the damage in all treated mice

combination-treated mice as compared with those seen in FGF2 or vehicle (4 and 3.4 vs. 2-fold increase vs. vehicle-treated mice, respectively $p<0.01$).

Taken together, these results suggest that VEGF may have an instructive role towards astroglial, oligodendroglial, and neuronal differentiation while FGF2 increases glial but not neuronal or oligodendroglial differentiation. Furthermore, it appears that the combination of VEGF and FGF2 does not significantly impact the rate of differentiation towards any of the neural fates as compared with VEGF alone.

Treatment Effects on Angiogenesis after Injury

At least part of the benefit afforded by VEGF or FGF2 could be attributed to their well-known effects on angiogenesis. To examine the effect of exogenous VEGF, FGF2, or their combination on angiogenesis, blood vessels were stained with an antibody against the endothelial marker CD31. A significant increase in the number of blood vessels was observed in the cortex around the lesion in all groups with the largest effects seen in animals treated with VEGF (3.3 vs. 1.4 or 1.9-fold increase vs. vehicle-treated mice, respectively) (Fig. 5) suggesting that treatment either protected existing blood vessels or led to a pro-angiogenic effect resulting in the formation of new blood vessels.

Discussion

The findings of the current study suggest that combining two potent inducers of neurogenesis and angiogenesis such as VEGF and FGF2 does not lead to additive effects on cell proliferation, neuronal differentiation, angiogenesis, or functional outcome after TBI. These results can be explained by exhaustion of the capacities for brain regeneration and may suggest the presence of a ceiling effect for inducing neurogenesis and angiogenesis after injury. Importantly, since both FGF2 and VEGF activate signaling through tyrosine kinase receptors, it is possible that the downstream signaling systems through phosphorylation of akt (Shore et al. 2004; Thau-Zuchman et al. 2010; Yun et al. 2010) or ERK may become saturated prohibiting further improvements. Nevertheless, we cannot exclude the possibility that combining growth and survival factors that act via different downstream signaling mechanisms such as signaling through Notch receptor pathway (Androutsellis-Theotokis et al. 2006) may still result in additive effects on neurogenesis and angiogenesis and would thus lead to significant functional gains.

Importantly, the rates of functional motor and sensory recovery following CHI were significantly higher in animals treated with VEGF and animals treated with VEGF–FGF combination therapy compared with FGF2 alone or vehicle, suggesting that the VEGF component of the combination therapy had a significant neuroprotective effect as evidenced by the early improvement in NSS and by the significantly lower lesion volume seen at 90 days post TBI. In our previous studies, we found an early increase in pAkt in the brain shortly after VEGF administration suggesting that the early neuroprotective effects of exogenous VEGF in TBI are probably mediated, at least in part, via induction of survival-signaling through pAkt and a reduction in apoptosis (Kilic et al. 2006; Parcellier et al. 2008; Shein et al. 2007; Thau-Zuchman et al. 2010). Cells in the SVZ continue to proliferate over long periods of time following ischemic lesions (Leker et al. 2007; Thored et al. 2007) and this appears to be also true for TBI as shown in the current work.

In conclusion, the present findings suggest that combining two potent inducers of neurogenesis and angiogenesis such as VEGF and FGF2 does not produce neither synergistic nor additive effects on cell proliferation, neuronal differentiation, angiogenesis, or functional outcome after TBI. For future experiments, we suggest to combine factors acting on different downstream signaling systems.

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Disclosure and Conflict of Interest All authors hereby declare they have no conflicts of interest.

References

Androutsellis-Theotokis A, Leker RR, Soldner F, Hoeppner DJ, Ravin R, Poser SW, Rueger MA, Bae SK, Kittappa R, McKay RD (2006) Notch signalling regulates stem cell numbers in vitro and in vivo. Nature 442(7104):823–826

Beauchamp K, Mutlak H, Smith WR, Shohami E, Stahel PF (2008) Pharmacology of traumatic brain injury: where is the "golden bullet"? Mol Med 14(11–12):731–740

Beni-Adani L, Gozes I, Cohen Y, Assaf Y, Steingart RA, Brenneman DE, Eizenberg O, Trembolver V, Shohami E (2001) A peptide derived from activity-dependent neuroprotective protein (ADNP) ameliorates injury response in closed head injury in mice. J Pharmacol Exp Ther 296(1):57–63

Chen Y, Constantini S, Trembovler V, Weinstock M, Shohami E (1996) An experimental model of closed head injury in mice: pathophysiology, histopathology, and cognitive deficits. J Neurotrauma 13 (10):557–568

Dore-Duffy P, Wang X, Mehedi A, Kreipke CW, Rafols JA (2007) Differential expression of capillary VEGF isoforms following traumatic brain injury. Neurol Res 29(4):395–403

Fabel K, Tam B, Kaufer D, Baiker A, Simmons N, Kuo CJ, Palmer TD (2003) VEGF is necessary for exercise-induced adult hippocampal neurogenesis. Eur J Neurosci 18(10):2803–2812

Flierl MA, Stahel PF, Beauchamp KM, Morgan SJ, Smith WR, Shohami E (2009) Mouse closed head injury model induced by a weight-drop device. Nat Protoc 4(9):1328–1337

Gage FH, Kempermann G, Palmer TD, Peterson DA, Ray J (1998) Multipotent progenitor cells in the adult dentate gyrus. J Neurobiol 36(2):249–266

Horner PJ, Palmer TD (2003) New roles for astrocytes: the nightlife of an 'astrocyte'. La vida loca! Trends. Neurosci 26(11):597–603

Jin K, Mao XO, Greenberg DA (2006) Vascular endothelial growth factor stimulates neurite outgrowth from cerebral cortical neurons via Rho kinase signaling. J Neurobiol 66(3):236–242

Kilic E, Kilic U, Wang Y, Bassetti CL, Marti HH, Hermann DM (2006) The phosphatidylinositol-3 kinase/Akt pathway mediates VEGF's neuroprotective activity and induces blood brain barrier permeability after focal cerebral ischemia. FASEB J 20(8):1185–1187

Kim JH, Chung YG, Kim CY, Kim HK, Lee HK (2004) Upregulation of VEGF and FGF2 in normal rat brain after experimental intraoperative radiation therapy. J Korean Med Sci 19(6):879–886

Krum JM, Rosenstein JM (1998) VEGF mRNA and its receptor flt-1 are expressed in reactive astrocytes following neural grafting and tumor cell implantation in the adult CNS. Exp Neurol 154(1):57–65

Lafuente JV, Argandona EG, Mitre B (2006) VEGFR-2 expression in brain injury: its distribution related to brain–blood barrier markers. J Neural Transm 113(4):487–496

Leker RR, Shohami E (2002) Cerebral ischemia and trauma—different etiologies yet similar mechanisms: neuroprotective opportunities. Brain Res Brain Res Rev 39(1):55–73

Leker RR, Soldner F, Velasco I, Gavin DK, Androutsellis-Theotokis A, McKay RD (2007) Long-lasting regeneration after ischemia in the cerebral cortex. Stroke 38(1):153–161

Nag S, Takahashi JL, Kilty DW (1997) Role of vascular endothelial growth factor in blood–brain barrier breakdown and angiogenesis in brain trauma. J Neuropathol Exp Neurol 56(8):912–921

Nakatomi H, Kuriu T, Okabe S, Yamamoto S, Hatano O, Kawahara N, Tamura A, Kirino T, Nakafuku M (2002) Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell 110(4):429–441

Nieto M, Schuurmans C, Britz O, Guillemot F (2001) Neural bHLH genes control the neuronal versus glial fate decision in cortical progenitors. Neuron 29(2):401–413

Palmer TD, Willhoite AR, Gage FH (2000) Vascular niche for adult hippocampal neurogenesis. J Comp Neurol 425(4):479–494

Papavassiliou E, Gogate N, Proescholdt M, Heiss JD, Walbridge S, Edwards NA, Oldfield EH, Merrill MJ (1997) Vascular endothelial growth factor (vascular permeability factor) expression in injured rat brain. J Neurosci Res 49(4):451–460

Parcellier A, Tintignac LA, Zhuravleva E, Hemmings BA (2008) PKB and the mitochondria: AKTing on apoptosis. Cell Signal 20(1):21–30

Shein NA, Tsenter J, Alexandrovich AG, Horowitz M, Shohami E (2007) Akt phosphorylation is required for heat acclimation-induced neuroprotection. J Neurochem 103(4):1523–1529

Shetty AK, Rao MS, Hattiangady B, Zaman V, Shetty GA (2004) Hippocampal neurotrophin levels after injury: relationship to the age of the hippocampus at the time of injury. J Neurosci Res 78(4):520–532

Shore PM, Jackson EK, Wisniewski SR, Clark RS, Adelson PD, Kochanek PM (2004) Vascular endothelial growth factor is increased in cerebrospinal fluid after traumatic brain injury in infants and children. Neurosurgery 54(3)):605–611; discussion 611-602

Skold MK, von Gertten C, Sandberg-Nordqvist AC, Mathiesen T, Holmin S (2005) VEGF and VEGF receptor expression after experimental brain contusion in rat. J Neurotrauma 22(3):353–367

Skold MK, Risling M, Holmin S (2006) Inhibition of vascular endothelial growth factor receptor 2 activity in experimental brain contusions aggravates injury outcome and leads to early increased neuronal and glial degeneration. Eur J Neurosci 23(1):21–34

Swanson RA, Morton MT, Tsao-Wu G, Savalos RA, Davidson C, Sharp FR (1990) A semiautomated method for measuring brain infarct volume. J Cereb Blood Flow Metab 10(2):290–293

Thau-Zuchman O, Shohami E, Alexandrovich AG, Leker RR (2010) Vascular endothelial growth factor increases neurogenesis after traumatic brain injury. J Cereb Blood Flow Metab 30(5):1008–1016

Thored P, Wood J, Arvidsson A, Cammenga J, Kokaia Z, Lindvall O (2007) Long-term neuroblast migration along blood vessels in an area with transient angiogenesis and increased vascularization after stroke. Stroke 38(11):3032–3039

Tsenter J, Beni-Adani L, Assaf Y, Alexandrovich AG, Trembovler V, Shohami E (2008) Dynamic changes in the recovery after traumatic brain injury in mice: effect of injury severity on T2-weighted MRI abnormalities, and motor and cognitive functions. J Neurotrauma 25(4):324–333

Wang WY, Dong JH, Liu X, Wang Y, Ying GX, Ni ZM, Zhou CF (2005) Vascular endothelial growth factor and its receptor Flk-1 are expressed in the hippocampus following entorhinal deafferentation. Neuroscience 134(4):1167–1178

Wang Y, Jin K, Mao XO, Xie L, Banwait S, Marti HH, Greenberg DA (2007) VEGF-overexpressing transgenic mice show enhanced post-ischemic neurogenesis and neuromigration. J Neurosci Res 85(4):740–747

Widenfalk J, Lipson A, Jubran M, Hofstetter C, Ebendal T, Cao Y, Olson L (2003) Vascular endothelial growth factor improves functional outcome and decreases secondary degeneration in experimental spinal cord contusion injury. Neuroscience 120(4):951–960

Xiao Z, Kong Y, Yang S, Li M, Wen J, Li L (2007) Upregulation of Flk-1 by bFGF via the ERK pathway is essential for VEGF-mediated promotion of neural stem cell proliferation. Cell Res 17(1):73–79

Yun YR, Won JE, Jeon E, Lee S, Kang W, Jo H, Jang JH, Shin US, Kim HW (2010) Fibroblast growth factors: biology, function, and application for tissue regeneration. J Tissue Eng 2010:218142