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Glycogen synthase kinase-3β inhibition enhances myelination in preterm newborns with intraventricular hemorrhage, but not recombinant Wnt3A

Preeti Dohare[a], Bokun Cheng[a], Ehsan Ahmed[a], Vivek Yadala[a], Pranav Singla[a], Sunisha Thomas[a], Robert Kayton[c], Zoltan Ungvari[d], Praveen Ballabh[a,b,*]

[a] Department of Pediatrics, Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[b] Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[c] Department of Physiology and Pharmacology, Oregon Health & Science University, Portland, Oregon 97239, USA
[d] Reynolds Oklahoma Center on Aging, Oklahoma University, OK, USA

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ABSTRACT

Intraventricular hemorrhage (IVH) in preterm infants results in reduced proliferation and maturation of oligodendrocyte progenitor cells (OPCs), and survivors exhibit reduced myelination and neurological deficits. Wnt signaling regulates OPC maturation and myelination in a context dependent manner. Herein, we hypothesized that the occurrence of IVH would downregulate Wnt signaling, and that activating Wnt signaling by GSK-3β inhibition or Wnt3A recombinant human protein (rh-Wnt3A) treatment might promote maturation of OPCs, myelination of the white matter, and neurological recovery in premature rabbits with IVH. These hypotheses were tested in autopsy samples from preterm infants and in a rabbit model of IVH. Induction of IVH reduced expressions of activated β-catenin, TCF-4, and Axin2 transcription factors in preterm newborns. Both AR-A014418 (ARA) and Wnt-3A treatment activated Wnt signaling. GSK-3β inhibition by intramuscular ARA treatment accelerated maturation of OPCs, myelination, and neurological recovery in preterm rabbits with IVH compared to vehicle controls. In contrast, intracerebroventricular rh-Wnt3A treatment failed to enhance myelination and neurological function in rabbits with IVH. ARA treatment reduced microglia infiltration and IL1β expression in rabbits with IVH relative to controls, whereas Wnt3A treatment elevated TNFα, IL1β, and IL6 expression without affecting microglia density. GSK-3β inhibition downregulated, while rh-Wnt3A treatment upregulated Notch signaling; and none of the two treatments affected the Sonic-Hedgehog pathway. The administration of ARA or rh-Wnt3A did not affect gliosis. The data suggest that GSK-3β inhibition promoted myelination by suppressing inflammation and Notch signaling; and Wnt3A treatment failed to enhance myelination because of its pro-inflammatory activity and synergy with Notch signaling. GSK-3β inhibitors might improve the neurological outcome of preterm infants with IVH.

1. Introduction

Intraventricular hemorrhage (IVH) remains the most common neurological complication of prematurity (Ballabh, 2010). Premature infants with IVH suffer from neurological consequences, including cerebral palsy, cognitive deficits, and intellectual disability (Ballabh, 2010). IVH inhibits proliferation and maturation of oligodendrocyte progenitor cells (OPCs), and thereby reduces myelination of the periventricular white matter in preterm newborns (Dummula et al., 2011). Currently, no therapeutic or preventive strategy exists to minimize white matter injury in premature infants with IVH. Wnt/β-catenin signaling has been linked with both neurodevelopmental and neurodegenerative disorders (Moon et al., 2004). Importantly, it regulates oligodendrogensis and myelination, however the underlying mechanisms are complex, context dependent, and much debated (Guo et al., 2015). Herein, we asked whether IVH would affect Wnt signaling and if so, whether activation of Wnt/β-catenin signaling pathway would enhance generation and maturation of oligodendrocyte progenitor cells (OPCs), and myelination in premature newborns with IVH.

The activation of Wnt/β-catenin pathway involves the binding of extracellular Wnt ligands to transmembrane fizzled and LRP5/6 receptors (Moon et al., 2004). This results in the dissociation of this destruction complex and consequent elevation in cytoplasmic β-catenin and its translocation in the nucleus to induce Wnt-target genes through

* Corresponding author at: Department of Pediatrics and Neuroscience, Division of Neonatology, The Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, NY 10461, USA.
E-mail address: Pballabh@msn.com (P. Ballabh).

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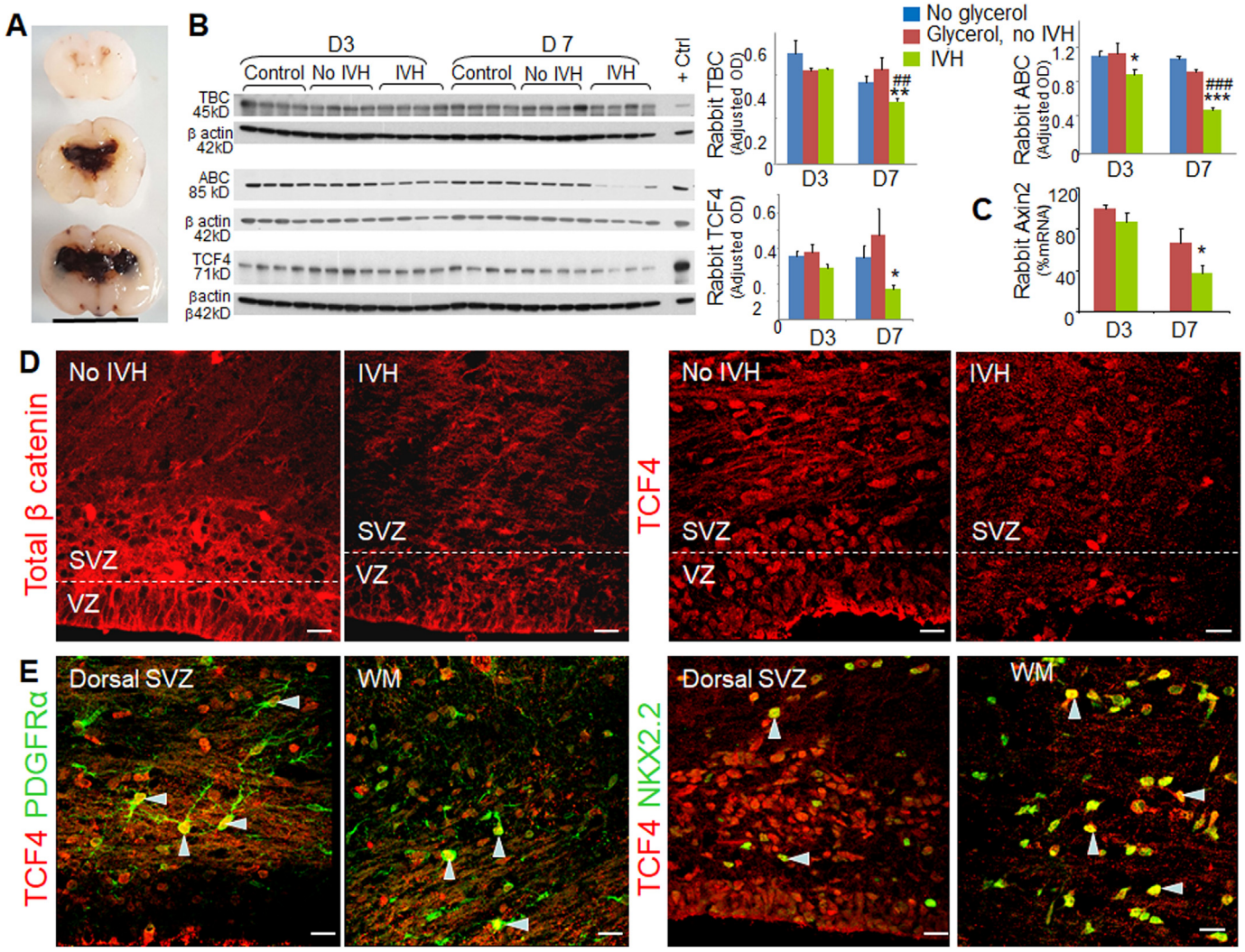

Fig. 1. IVH reduced active β-catenin (ABC) and TCF4 in preterm rabbits. A) Coronal brain slice from the fronto-parietal lobe of E29 rabbit pups which show slit like ventricles in pup without IVH (upper panel) and moderate to severe IVH resulting in fusion of the lateral ventricles (middle and lower panel). Scale bar, 1 cm. B) Representative Western blot analyses for total β-catenin (TBC), activated β-catenin (ABC) and TCF4 on brain homogenates of preterm rabbits with and without IVH at D3 and D7. The bar charts are mean ± s.e.m. ($n = 5$ each). Values were normalized to β actin levels. TBC levels were reduced in pups with IVH compared to both glycerol-treated and untreated controls without IVH at D7. ABC was reduced in rabbits with IVH compared to both glycerol and no glycerol controls without IVH at D7. ABC was also reduced in pups with IVH compared to glycerol treated controls without IVH at D3. TCF4 was also reduced in rabbits with IVH compared to controls without IVH at D7. C) mRNA expressions of Axin2 was reduced in rabbits with IVH compared to controls without IVH at D7. D) Representative immunofluorescence of cryosections from E29 rabbit pups with and without at D7 (as indicated) labeled with β-catenin and TCF4 specific antibodies. Note reduced expression of β-catenin and TCF4 in the VZ and SVZ of dorsal telencephalon of rabbits with IVH compared to controls without IVH. E) Cryosections were stained with TCF4 in combination with PDGFRα or Nkx2.2 specific antibodies. TCF4 immunoreactivity co-localized with PDGFRα extensively in the SVZ of dorsal telencephalon (arrowhead). TCF4 signals also overlapped with Nkx2.2 reactivity (arrowhead). Scale bar, 20 μm. *P < .05, **P < .01, ***P < .001 glycerol treated no IVH controls vs. pups with IVH. ##P < .01, ###P < .001 pups not treated with glycerol vs. pups with IVH. VZ, ventricular zone; SVZ, subventricular zone.

TCF4 activation (TCF7L2). The intracellular β-catenin destruction complex consists of Axin, adenomatous polyposis coli (APC), glycogen synthase kinase 3β (GSK3β), and casein kinase 1. Indeed, Wnt signaling is regulated at multiple levels; and GSK-3β inhibition has been employed in a number studies to activate Wnt-β-catenin signaling pathways (Moon et al., 2004; Rockenstein et al., 2007).

The prevailing notion was that the activation of Wnt/β-catenin signaling inhibits OPC differentiation and contributes to myelination failure. Accordingly, administration of Wnt antagonist (Shimizu et al., 2005) and inactivation of Wnt signaling in mouse model increases OPC generation (Langseth et al., 2010; Ye et al., 2009); and stabilization of axin2 by tankyrase inhibition, or downregulation of β-catenin enhances OPC maturation and myelination (Fancy et al., 2011). Conditional ablation of APC from oligodendroglial lineage inhibits maturation of OPCs through both β catenin dependent and independent mechanisms (Lang et al., 2013). However, these studies have been challenged by recent reports, which show that activation of canonical Wnt signaling in culture and in vivo experiments promotes oligodendrogenesis (Azim and Butt, 2011; Ortega et al., 2013). Likewise, Wnt signaling activation by GSK3 β inhibition increases OPC specification and differentiation in the dorsal domain of the subventricular zone (Azim et al., 2017; Azim and Butt, 2011; Azim et al., 2014). Hence, a refined concept has been proposed that Wnt signaling plays distinct roles in OPC proliferation, maturation, and myelination in a context dependent manner.

Glycogen synthase kinases are serine/threonine kinases which play crucial roles in both neurogenesis as well as gliogenesis (Hur and Zhou, 2010). Glycogen synthase kinases-3β (GSK-3 β) is a dynamic enzyme which controls various signaling pathways regulating oligodendrogenesis, including TGFβ, Shh, Notch, and Wnt pathways (Hur and Zhou, 2010). In addition, it participates in signaling pathways that control