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Research Report

Diazoxide promotes oligodendrocyte differentiation in neonatal brain in normoxia and chronic sublethal hypoxia

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ABSTRACT

Periventricular white matter injury (PWMI) is the most common cause of brain injury in preterm infants. It is believed that loss of late oligodendrocyte progenitor cells (OPCs) and disrupted maturation of oligodendrocytes contributes to defective myelination in PWMI. At present, no clinically approved drugs are available for treating PWMI. Previously, we found that diazoxide promotes myelination and attenuates brain injury in the chronic sublethal hypoxia model of PWMI. In this study, we investigated the mechanisms by which diazoxide promotes myelination. We observed that diazoxide increases the ratio of differentiated oligodendrocytes in the cerebral white matter, promotes the expression of differentiation-associated transcriptional factors Nkx2.2 and Sox10, and increases the expression of myelin genes CNP and MBP. These results show that diazoxide promotes oligodendrocyte differentiation in the developing brain.

1. Introduction

Periventricular white matter injury (PWMI) is the most common cause of brain injury in preterm infants (Back and Rosenberg, 2014; Salmaso et al., 2014). The period of highest risk for PWMI is 23–32 weeks postconception age, and the risk factors of PWMI include ischemia, hypoxia and inflammation (Borch and Greisen, 1998; Glass et al., 2008; Volpe, 2009). Infants with PWMI are at risk for long-term neurological deficits, including cerebral palsy, cognitive and visual deficits, and learning disabilities (Anderson and Doyle, 2003; Anderson et al., 2011; Johnson et al., 2010). Unfortunately, there are no specific treatments for PWMI at present.

There are two major forms of PWMI that include diffuse white matter injury and focal cystic necrotic lesions (Back and Rivkees, 2004; Back and Rosenberg, 2014; Volpe, 2009). Of these types, diffuse white matter injury is the most common form and is characterized by global hypomyelination (Counsell et al., 2003; Hamrick et al., 2004). The period of greatest risk for PWMI is coincident with the onset of oligodendrocyte differentiation and early myelination in the brain when oligodendrocyte progenitor cells (OPCs) and immature oligodendrocytes predominate. It is believed that PWMI involves depletion of late OPCs and disrupted maturation of oligodendrocytes, which leads to impaired myelination (Segovia et al., 2008). Supporting this notion, hypoxia,

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ischemia, and cytokines induce death of premature oligodendrocytes and cause impaired myelination (Karadottir et al., 2005; Khwaja and Volpe, 2008).

Several lines of evidence suggest that OPCs can regenerate and be recruited to white matter lesions (Aguirre et al., 2007; Frost et al., 2003). However, the differentiation of OPCs into myelinating oligodendrocytes may be impaired (Jablonska et al., 2012; Segovia et al., 2008), contributing to white matter injury (Alix et al., 2012; Chang et al., 2002; Kuhlmann et al., 2009). The ability to stimulate the differentiation of OPCs into myelinating oligodendrocytes is therefore an attractive approach for developing therapies for PMWI. Few agents, however, are known to stimulate oligodendrocyte differentiation in vivo (Deshmukh et al., 2013; Fancy et al., 2011; Scafidi et al., 2014).

Recently, we discovered that diazoxide promotes myelination in the neonatal brain and attenuates hypoxia-induced brain injury in neonatal mice (Fogal et al., 2010). Diazoxide is an ATP-sensitive potassium channel activator that is approved by the US Food and Drug Administration (FDA) for the treatment of hyperinsulinism in infants and has a favorable safety profile (Stanley, 2006; US Food and Drug Administration, 2014). Previous studies demonstrated that diazoxide has neuroprotective effects in ischemia and hypoxia as well (Domoki et al., 1999; Shake et al., 2001). The mechanisms underlying the beneficial effects of diazoxide on myelination, though, remain unknown.

In this study, we examined the effects of diazoxide on oligodendrocyte proliferation and differentiation using the chronic sub-lethal hypoxia (CSH) model of PWMI (Back and Rivkees, 2004; Fogal et al., 2010; Jablonska et al., 2012; Scafidi et al., 2009). We now report that diazoxide promotes the differentiation of oligodendrocytes.

2. Results

2.1. *Influence of diazoxide on oligodendrocyte proliferation in cerebral white matter*

We first examined the effect of diazoxide on the proliferation rate of OPCs. 5-bromo-2′-deoxyuridine (BrdU) labeling was performed and followed by immunofluorescence staining with anti-BrdU and anti-Olig2 antibodies (Fig. 1). Short-term diazoxide treatment from P3 to P7 did not affect the rates of oligodendrocyte proliferation in the external capsule in normoxic or hypoxic conditions (Fig. 1E). In addition, the diazoxide-treated mice had a similar density of oligodendrocytes in the external capsule as vehicle-controls (Fig. 1F). These results indicate that diazoxide does not promote proliferation, nor increase the number of oligodendrocytes in the cerebral white matter during neonatal stages.

We also examined the effect of hypoxia on oligodendrocyte proliferation. Short-term hypoxia treatment significantly reduced the proliferation rate of oligodendrocytes in the external capsule by 43.19±12.83% (Fig. 1E). The density of oligodendrocytes was comparable between normoxia- and hypoxia-reared mice at P7 (Fig. 1F), and we did not detect increased apoptosis in this area (Supplementary Fig. 1). These data indicate that the hypoxia suppresses the proliferation of OPCs in the cerebral white matter.

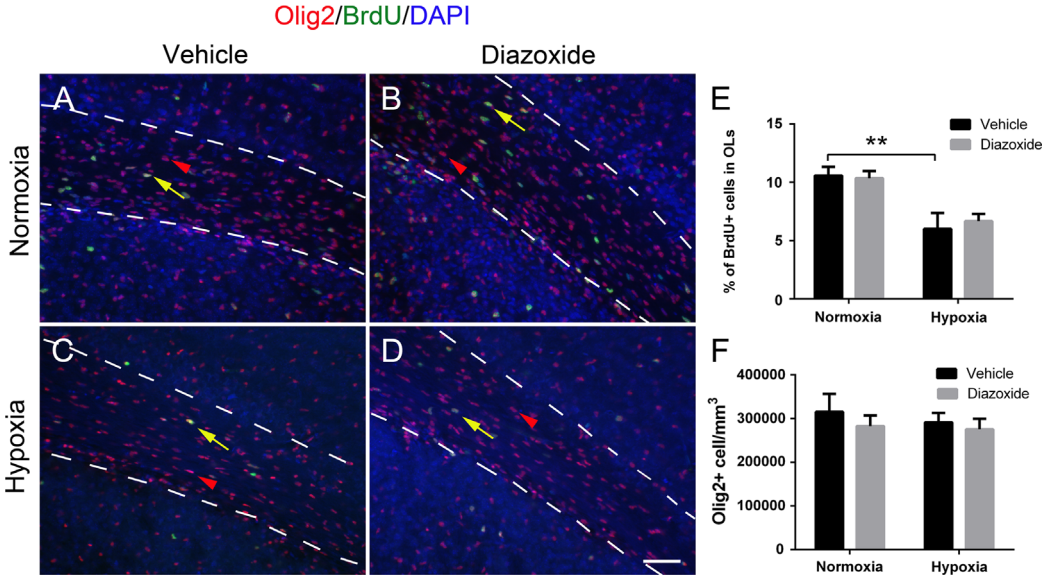

Fig. 1 – Effects of diazoxide and hypoxia on oligodendrocyte proliferation in the external capsule. (A–D) Confocal images of P7 cerebral sections from normoxia and hypoxia reared mice with treatment of vehicle or diazoxide from P3 to P7. Tissue sections were stained with anti-Olig2 (red) and anti-BrdU (green) antibodies and nuclei were counterstained with DAPI (blue). Dashed lines mark the external capsule. Arrows indicate anti-Olig2 and anti-BrdU double-labeled proliferating oligodendrocytes; arrow heads indicate Olig2+ cells. Scale bar represents 50 μm. (E) Quantification of percentage of Olig2+ cells that are co-labeled with BrdU in the external capsule. OL, oligodendrocyte. (F) Quantification of the density of oligodendrocytes in the external capsule. $n_{normoxia}=3$, $n_{hypoxia}=3$. Two way ANOVA with uncorrected Fisher's LSD, **, $p<0.01$. Error bars represent standard error mean (SEM).