

Identification of Diazoxide Analogues that Stimulate Oligodendrocyte Proliferation

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Abstract

Introduction and Objectives: Up to 30% of low birth weight preterm infants manifest some form of periventricular white matter injury (PWMI) making it the most common form of brain injury affecting premature infants. It is believed that loss of oligodendrocyte progenitor cells (OPCs), which are proliferative cells that develop into myelinating OLs, plays a major role in PWMI causation. Presently, few pharmacological approaches specifically target OPCs resulting in increased proliferation of these cells and increased brain myelination. We discovered that diazoxide (DZ), an activator of ATP-sensitive potassium channels (KATP), promotes OPC proliferation and attenuated hypoxia induced brain injury in neonatal mice. Out of 610 Diazoxide derivatives, compound K261-0298 (ChemDiv) was identified as the most potent stimulator of myelination among our lead compounds.

Methods: Using in vivo toxicology studies we assessed the approximate LD50 for K261-0298 in a stepwise approach. Next compound kinetics were examined via liquid chromatography/mass spectrophotometry (LC/MS) on mice blood samples. Myelination studies in newborn mice reared in room air were performed to determine markers of myelination and oligodendrocyte proliferation. Hypoxia exposure with follow-up ventricular area measurements were conducted to determine effect on ventriculomegaly in hypoxia exposed mice.

Results: In the juvenile mice that were treated from P7-P17 or adults treated from P40-P50, no abnormalities were seen. Blood samples from male and female were collected prior to dosing and after 0.5, 2, 4, 8 and 24 hours with 100 mg/kg of K261-0298. Data from serum LC-MS/MS revealed that peak drug levels were 21.5 +/- 2.3 M and the circulating half-life was 2.2 +/- 0.2 hrs. Tissue slices were stained for markers of myelination (MBP) and oligodendrocyte development (O1, O4). This analysis revealed a 27 ± 4% increase in MBP labeling, a 44 ± 4% decrease in O4 labeling, and a 26 ± 5% increase in O1 labeling vs. vehicle (n=6; p<0.02; ANOVA). These data suggest that there is increased maturation of oligodendrocyte lineage favoring the development of O1-positive myelinating oligodendrocytes. At the end of the treatment period mice were examined for ventriculomegaly, as reported. We observed a marked reduction in ventriculomegaly in the K261-0298 (0.0054 +/- 0.00013 m2) vs. vehicle-treated (0.0156 +/- 0.0039 m2) mice (n= 4) per treatment, p<0.03).

Conclusions-Implications: Collectively, we show that we identified a compound that is non-toxic, has favorable pharmacokinetic properties, promotes the development of myelinating

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oligodendrocytes, and stimulates myelination in vivo and in vitro. The next phase will be focused on testing K261-0298 in two different models of white matter injury (hypoxia and LPS) to establish the ideal dose and the effects of 298. Extensive toxicology studies will also be done to ensure safety for potential future human trials.

Identification of Diazoxide Analogues that Stimulate Oligodendrocyte Proliferation

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ABSTRACT

Up to 30% of low birth weight preterm infants manifest some form of periventricular white matter injury (PVM) making it the most common form of brain injury affecting premature infants. PVM includes a spectrum of brain injury ranging from diffuse white matter disease to focal necrosis. PVM is associated with significant morbidity, as affected individuals may have profound intellectual impairment and cerebral palsy.

Oligodendrocytes (OLs) are the myelinating cells of the central nervous system and play a critical role in white matter formation. It is believed that loss of oligodendrocyte progenitor cells (OPCs), which are proliferative cells that develop into myelinating OLs, plays a major role in PVM causation. Presently, few pharmacological approaches specifically target OPCs resulting in increased proliferation of these cells and increased brain myelination.

We discovered that diazoxide (DZ), an activator of ATP-sensitive potassium channels (K_{ATP}), promotes OPC proliferation and attenuated hypoxia induced brain injury in neonatal mice. To identify other potential therapeutic agents that may stimulate OPCs, we assessed the influence of 610 derivative compounds of diazoxide on OPC proliferation in vitro. Using high throughput screening, we identified 25 compounds that stimulated OPC proliferation, four of which, that were significantly more potent than DZ. Next, we identified the compound K261-0298 (ChemIDw) as the most potent stimulator of myelination among our lead compounds. This compound was tested in short-term and long-term toxicology studies in neonatal mice and was non-toxic at doses up to 100 mg/kg. In vivo, this compound stimulated myelination in neonatal mice reared in either room air or hypoxia. These data show that we identified a novel compound that can stimulate myelination in the developing brain.

BACKGROUND

Periventricular White Matter Injury (PVM) is the most common form of brain injury affecting premature infants. PVM is associated with attention, behavioral, and socialization deficits.

Loss of Oligodendrocyte Progenitor Cells (OPCs) is believed to play a role in causation of PVM, stimulating OPC proliferation may be a therapeutic strategy to treat PVM.

We discovered that diazoxide, which is an activator of ATP-sensitive potassium channels (K_{ATP}), promotes OPC proliferation in vitro and attenuates hypoxia-induced brain injury in neonatal mice.

Diazoxide

↓

610 Derivatives

↓

25 Stimulated OPC Proliferation

↓

4 More Potent than Diazoxide

↓

K261-0298 Most Potent Stimulator

HYPOTHESIS: K261-0298, a diazoxide derivative, promotes oligodendrocyte cell proliferation and leads to increased myelin basic protein (MBP) expression.

METHODS & RESULTS

In Vivo Toxicology Studies

We assessed the approximate LD_{50} for these new compounds with a step wise approach using doses 5, 50, 100, and 400 mg/kg. The final concentration of DMSO was 4% and this was used as the vehicle control for all in vivo experiments. Mice were treated with a single dose. The health and weights of animals were monitored daily over the following 2 weeks. Necropsies and histopathology studies were performed on 5 controls and 5 K261-0298-treated mice for both ages, juvenile and adult. In the juvenile mice that were treated from P7-P17 or adults treated from P40-P50, no abnormalities were seen.

Hypoxia Exposure/Ventricular Area Measurements

Mice were exposed to low or normal oxygen levels from P2-P12. At the end of treatment (P12), mice were euthanized by CO_2 and perfused with PBS and 4% PFA before tissue collection. Brains were collected and post-fixed. Free-floating thick coronal brain sections (100 μ m) were cut in a Vibratome. Sections from the start of the corpus callosum to the rostral end of the third ventricle were collected for IHC and ventricular area measurements.

Myelination Studies in Newborn Mice in Room Air

Pups were examined to assess effects on myelination, as reported by us. C57BL/6 mice were reared in room air from P2 to P12, and treated with the compound or vehicle. Daily injections of K261-0298 (100 mg/kg) were given, as above. Tissue slices were stained for markers of myelination (MBP) and oligodendrocyte development (O1, O4).

K261 Increases Myelination in Room Air

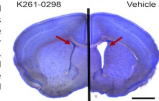
Myelin basic protein (MBP), O4 and O1 labeling (green) of coronal forebrain at level of corpus callosum (cc) of newborn mice treated with vehicle or drug (K261-298) from P2 - P12 in room air. Data shown are representative of at least 6 pups per treatment group. cc, corpus callosum. Scale bar = 100 μ m. Left panels show whole brain slices, with inset box showing areas of staining. This analysis revealed a 27 \pm 4% increase in MBP labeling, a 44 \pm 4% decrease in O4 labeling, and a 26 \pm 5% increase in O1 labeling vs. vehicle (n = 6; p<0.02; ANOVA). These data suggest that there is increased maturation of oligodendrocyte lineage favoring the development of O1-positive myelinating oligodendrocytes.

Liquid Chromatography/Mass Spectrophotometry (LC/MS)

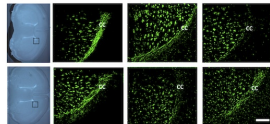
Next compound kinetics were examined. Blood samples from male and female were collected prior to dosing and after 0.5, 2, 4, 8 and 24 hours with 100 mg/kg of K261-0298. Serum concentrations of K261-0298 were determined by LC-MS/MS. These data revealed that peak drug levels were 21.5 +/- 2.3 mM and the circulating half-life was 2.2 +/- 0.2 hrs.

K261 Lead to Reduction in Ventriculomegaly in Hypoxia-Exposed Mice

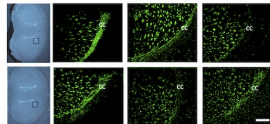
Nissl-stained, hemi-coronal sections at the same brain levels shows. Data shown are representative of 4 pups per treatment group (100 mg/kg/d, P2-P12). Arrow depicts lateral ventricle. Note smaller ventricle size in the K261-0298 treated pup. Scale bar = 500 μ m.



Vehicle



298



CONCLUSIONS

Collectively, we show that we identified a compound that is non-toxic, has favorable pharmacokinetic properties, promotes the development of myelinating oligodendrocytes, and stimulates myelination in vivo and in vitro.

FUTURE DIRECTIONS

The next phase will be focused on testing K261-0298 in two different models of white matter injury (hypoxia and LPS) to establish the ideal dose and the effects of 298. Extensive toxicology studies will also be done to ensure safety for potential future human trials.