Experimental Neurology 309 (2018) 1–13

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

The G2019S mutation in LRRK2 imparts resiliency to kinase inhibition

Kaela Kelly[a], Shijie Wang[a], Ravindra Boddu[b], Zhiyong Liu[a], Omar Moukha-Chafiq[c], Corinne Augelli-Szafran[c], Andrew B. West[a,*]

[a] Center for Neurodegeneration and Experimental Therapeutics, Department of Neurology, University of Alabama at Birmingham, Birmingham, AL 35233, USA
[b] Division of Nephrology, Nephrology Research and Training Center, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35233, USA
[c] Southern Research, Birmingham, AL, USA

ARTICLE INFO

Keywords:
Park8
Dardarin
Pharmacodynamics
Pharmacokinetics
Small-molecule inhibitor

ABSTRACT

The G2019S mutation in *LRRK2* is one of the most common known genetic causes of neurodegeneration and Parkinson disease (PD). *LRRK2* mutations are thought to enhance LRRK2 kinase activity. Efficacious small molecule LRRK2 kinase inhibitors with favorable drug properties have recently been developed for pre-clinical studies in rodent models, and inhibitors have advanced to safety trials in humans. Rats that express human G2019S-LRRK2 protein and G2019S-*LRRK2* knock-in mice provide newly characterized models to better understand the ostensible target for inhibitors. Herein, we explore the relationships between LRRK2 kinase inhibition in the brain and the periphery to establish the link between LRRK2 kinase activity and protein stability, induction of lysosomal defects in kidney and lung, and how G2019S-LRRK2 expression impacts these phenotypes. Using a novel ultra-sensitive scalable assay based on protein capillary electrophoresis with LRRK2 kinase inhibitors included in-diet, G2019S-LRRK2 protein was resilient to inhibition compared to wild-type (WT)-LRRK2 protein, particularly in the brain. Whereas WT-LRRK2 kinase activity could be completed blocked without lowering LRRK2 protein levels, higher inhibitor concentrations were necessary to fully reduce G2019S-LRRK2 activity. G2019S-LRRK2 expression afforded robust protection from inhibitor-induced kidney lysosomal defects, suggesting a gain-of-function for the mutation in this phenotype. In rodents treated with inhibitors, parallel measurements of phospho-Rab10 revealed a poor correlation to phospho-LRRK2, likely due to cells that express Rab10 but poorly express LRRK2 in heterogenous tissues and cell isolates. In summary, our results highlight several challenges associated with the inhibition of the G2019S-LRRK2 kinase that might be considered in initial clinical efforts.

1. Introduction

The *leucine-rich repeat kinase 2* gene encodes LRRK2 protein that is expressed primarily in circulating leukocytes, kidney, lung, and the brain in humans (West 2017). Genetic studies show that the pathogenic G2019S mutation in the LRRK2 kinase domain is one of the most frequent known genetic causes of neurodegeneration (Trinh et al. 2014). Initial *in vitro* studies in transfected cell lines revealed that G2019S-LRRK2 increased autophosphorylation activities as well as LRRK2 kinase activity towards generic peptide substrates, usually ~2–5 fold over endogenous wild-type (WT)-LRRK2. Analyses of LRRK2 protein harbored in extracellular exosomes purified from urine from LRRK2 mutation carriers with Parkinson's disease (PD) also suggests a similar effect on LRRK2 autophosphorylation (Fraser et al., 2016a; Wang et al. 2017). Emerging evidence suggests that LRRK2 autophosphorylation or expression may be likewise increased in a proportion of idiopathic PD (Bliederhaeuser et al. 2016; Cook et al. 2017). Toxicity associated with G2019S-LRRK2 expression has been demonstrated in multiple models, for example viral-expression systems, to depend on LRRK2 kinase activity (Dusonchet et al. 2011; Greggio et al. 2006; Lee et al. 2010; Tsika et al. 2015). As such, intensive efforts are devoted towards the development of LRRK2 kinase inhibitors for the treatment of LRRK2-linked PD (West 2017). Safety trials are underway with several LRRK2 kinase inhibitors of as-yet unknown identity (Hyland and Warners 2017).

The G2019S mutation in LRRK2 protein alters the conserved DYG motif to DYS in the kinase activation loop, plausibly affecting metal binding and flexibility required for kinase activation (Nolen et al. 2004). While there is no high-resolution structure available for the

Abbreviations: LRRK2, Leucine-rich repeat kinase 2; PD, Parkinson disease; ATP, adenosine-tri phosphate; DTT, dithiothreitol; HSC70, Heat-shock cognate 70; Rab, Ras gene from rat brain, or Ras-related in brain

* Corresponding author at: 1719 6th Ave. S., Birmingham, AL 35294, USA.
E-mail address: abwest@uab.edu (A.B. West).

https://doi.org/10.1016/j.expneurol.2018.07.012
Received 27 May 2018; Received in revised form 18 July 2018; Accepted 21 July 2018
Available online 24 July 2018
0014-4886/ © 2018 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/BY/4.0/).

LRRK2 kinase domain from higher-order eukaryotes, we previously used a library of thousands of ATP-competitive molecules to probe the ATP-binding pocket of WT- and G2019S-LRRK2 and identified molecules that could preferentially inhibit G2019S-LRRK2 *versus* WT-LRRK2 (Liu et al. 2014). Notably, several structurally distinct small molecule scaffolds have been described with very high specificity for LRRK2, where only weak binding to other protein kinases could be detected. We have attributed this property of some LRRK2 kinase inhibitors to the unique ATP-pocket and amino acid composition in human LRRK2 (Liu et al. 2014). Among ATP-competitive LRRK2 small molecule kinase inhibitors, the molecules MLi2 and PF-360 show low to sub-nanomolar binding *in vitro* and have outstanding selectivity profiles in blocking only LRRK2 kinase activity at lower concentrations out of hundreds of other kinases screened (Fell et al. 2015; Henderson et al. 2015; West 2015).

To facilitate the development of successful LRRK2-targeting therapeutics, rats that express human G2019S-LRRK2 as well as mice with the mutation knocked into the genome have been developed (Daher et al. 2015; Volta et al. 2017). These rodent models together with potent small molecule inhibitors provide an excellent opportunity to explore pharmacodynamic responses related to LRRK2 kinase inhibition both in the brain and periphery. Some *in vivo* activity profiles have been reported in WT mice for MLi2 and in WT rats for PF-360 in separate studies (Andersen et al. 2018; Baptista et al. 2015; Fell et al. 2015; Scott et al. 2017), but LRRK2 inhibition profiles have been poorly described in the context of G2019S-LRRK2 expression. In rats and non-human primates, oral-dosing strategies that result in brief periods of time with high concentrations of LRRK2 kinase inhibitors result in mild lysosomal alterations in lung and kidney tissue, partly resembling LRRK2 knockout rodents (Baptista et al. 2013; Fuji et al. 2015). These phenotypes related to lysosome dysfunction have proved difficult to quantitatively measure in past studies. Other studies in LRRK2 knockout mice hypothesize LRRK2-linked disease may relate better to loss-of-function phenotypes associated with LRRK2 mutations rather than gain-of-function phenotypes, as LRRK2 knockout mice may mimic some aspects of mice with LRRK2 mutations (Giaime et al. 2017). Further, some *in vitro* studies suggest that LRRK2 kinase activity controls LRRK2 protein levels (Skibinski et al. 2014), further obscuring the relationship between LRRK2 kinase inhibition and disease.

To explore these questions, we combine the most potent described LRRK2 tool kinase inhibitors MLi2 and PF-360 to probe LRRK2 kinase inhibition in the brain and periphery at different concentrations of compound in both WT and G2019S-LRRK2 expressing rats and mice. In deducing dose-response relationships for both LRRK2 inhibition and the development of potentially adverse phenotypes in the kidney and lung, we utilize in-diet drug regimens for both molecules able to stably sustain selected concentrations of compound between ~1 to ~100 nm in the brain and periphery. Further, we use a highly quantitative protein assay capable of measuring phospho-LRRK2 (pS935-LRRK2) in tissues and cells in the presence of strong detergents like sodium-dodecyl sulfate (SDS) and reducing agents like dithiothreitol (DTT) to ensure all LRRK2 protein is captured for analysis. Despite past reports, there is not a direct relationship between LRRK2 kinase activity and LRRK2 protein levels. Our observations in rats and mice expressing G2019S-LRRK2 demonstrate an unexpected resiliency of G2019S-LRRK2 protein to inhibition at controlled concentrations of drug. Tissue-specific effects add further complexity to G2019S-LRRK2 resiliency, with G2019S-LRRK2 in the brain more resilient than in the kidney or lung in both mice and rats. Inconsistent with a loss-of-function hypothesis for G2019S-LRRK2, mutant LRRK2 expression offers robust protection from lysosomal defects caused by higher concentrations of kinase inhibitors. Collectively, these results highlight some potentially unexpected challenges in the therapeutic targeting of G2019S-LRRK2 as well as provide additional support for a kinase-activation hypothesis for the G2019S mutation in PD.

2. Material and methods

2.1. In vitro assessment of LRRK2 kinase activity

Full length, recombinant human WT-LRRK2 (A15197/1779572) and G2019S-LRRK2 (A15200/1524191) kinase activity was measured using LanthaScreen kinase assays (ThermoFisher) as well as AlphaScreen assays (PerkinElmer) as previously described (Liu et al. 2014).

2.2. In silico docking models

The LRRK2 ATP binding pocket was constructed based on the MST3 structure (4u8z) using homologue modeling on SWISS-MODEL, as described (Henderson et al. 2015). Molecule substitutions were created with Pymol Bulider script, and amino-acid substitutions made with the Mutagenesis script. Docking models were generated using iGEM dock.

2.3. Compounds

MLi2 and PF-360 were synthesized in-house. Novel synthetic pathways are provided in Supplemental Files 1 and 2. Medicated chows were formulated at Research Diets.

2.4. Animals

All animal protocols were approved by local Animal Care and Use Committees accredited by the AAALAC. Both male and female rodents, aged 8–12 weeks were used in this study. All rodents were housed on a 12 h light/dark cycle and given free access to food and water. Consumption of chow was monitored daily and body weight regularly measured. G2019S-LRRK2 human BAC rats (Sprague-Dawley, Taconic), LRRK2 knockout rats (Long-Evans, Sage), and G2019S-LRRK2 knock-in mice (C57Bl6, Taconic), as well as non-transgenic littermates were used in this study. Approximately equal numbers of males and females compose all experiments and observations.

2.5. Pharmacokinetics

Male Sprague-Dawley rats (n = 3 for all observations) were used with IV doses at 3 mg kg^{-1}, with blood sampling at 5 min, 15 min, 30 min, 1, 2, 4, 8, and 24 h. PO dose was at 5 mg kg^{-1} with blood sampling at 15 min, 30 min, 1, 2, 4, 8, and 24 h. No clinical symptoms were observed during the experiments. Samples were analyzed by HPLC. Microsomes were prepared from macaque liver and included, with or without NADPH, at 0.5 mg mL^{-1} (w/v). The final concentrations of test compounds in microsome stability assays were 2 μM.

2.6. Mass spectrometry

Compound standards included 50% acetonitrile in water with known concentrations of compound (1–10,000 ng mL^{-1}) combined with plasma (mouse or rat) obtained on the same day of analysis. Standards and experimental solutions were analyzed on an Applied Biosystems Sciex Triple Quad 5500, with online LC-30 CE and Phenomenex Synergi 2.5 μm Polar-RP 3 × 50 mm columns. Mobile phase included 5% acetonitrile in water with 0.1% Formic acid, and a solution of 95% acetonitrile in water with 0.1% formic acid.

2.7. Tissue and cell lysates

Rat and mouse tissue was collected following transcardiac perfusion of cold PBS. Tissues were homogenized by sonication in a RIPA lysis buffer consisting of 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton, 0.1% SDS supplemented with 1× protease inhibitors (Fisher) and phosphatase inhibitors (Roche). Whole blood was collected into

Download English Version:

https://daneshyari.com/en/article/8684516

Download Persian Version:

https://daneshyari.com/article/8684516

Daneshyari.com