Proposed Therapies for Pantothenate-Kinase-Associated Neurodegeneration

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Journal of Experimental Neuroscience
Volume 13: 1–3

DOI: 10.1177/1179069519851118
SAGE

ABSTRACT: Multiple approaches to therapy have been proposed for the rare inherited neurodegenerative disease associated with mutations in the *PANK2* gene, called pantothenate-kinase-associated neurodegeneration (PKAN). Penetration of the blood-brain barrier for treatment of a central nervous system (CNS) disorder is a major challenge in drug discovery. Evaluation of the biochemistry and medicinal chemistry of the proposed therapies reveals potential liabilities among several compounds under consideration for clinical development.

KEYWORDS: Pantothenate, PKAN, neurodegeneration, coenzyme A, CNS therapy

RECEIVED: April 19, 2019. **ACCEPTED:** April 24, 2019.

TYPE: Commentary

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The author receives funding for research from a Sponsored Research Agreement with CoA Therapeutics, Inc, a subsidiary of BridgeBio LLC, and the American Syrian Lebanese Associated Charities (ALSAC).

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The author is an inventor on a pending patent application (PCT/US17/39037) "Small molecule modulators of pantothenate kinases" held by St Jude Children's Research Hospital that covers the pantazine chemical series discussed in this article.

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COMMENT ON: Sharma LK, Subramanian C, Yun MK, et al. A therapeutic approach to pantothenate kinase associated neurodegeneration. *Nat Commun*. 2018;9:4399. doi:10.1038/s41467-018-06703-2. PubMed PMID:30352999; PubMed Central PMCID:PMC6199309. https://www.ncbi.nlm.nih.gov/pubmed/30352999

Pantothenate-kinase-associated neurodegeneration (PKAN) is an inherited disease caused by *PANK2* gene mutations[1] that are thought to result in the reduction of cellular coenzyme A (CoA). Patients with PKAN exhibit a variety of symptoms, including dystonia, rigidity, bradykinesia, spasticity, difficulty swallowing and speaking, shortened lifespan, and sometimes cognitive and visual impairment.[2] The clinical symptoms are often associated with an accumulation of iron in the brain and postmortem pathology indicates an enrichment of ischemic foci in the globus pallidus,[3] implicating an interruption of oxidative metabolism in the central nervous system (CNS). CoA is cell autonomous and thus any effective PKAN therapy must penetrate both cellular membranes and the blood-brain barrier (BBB). One of the first ideas was to treat patients with pantothenate in an attempt to raise CoA synthesis by increasing substrate supply. Although it remains possible that high-dose pantothenate over extended periods may be useful in reducing the symptoms,[4] the strong feedback inhibition of the pantothenate kinases (PANKs) means that there is little to no increase in tissue CoA levels in animals treated with high-dose pantothenate.[5]

Three different approaches to PKAN therapy have been proposed that are designed to bypass the *PANK2* genetic defect by supplying a CoA biosynthetic pathway intermediate downstream of PANK. Phosphopantothenate is the product of PANK but cannot cross cell membranes due to its charged nature. Fosmetpantotenate was designed as a prodrug to deliver phosphopantothenate to cells and elevate intracellular CoA.[6] The charged moieties on phosphopantothenate are chemically masked by covalent modification with hydrophobic groups to promote penetration across cellular membranes. The synthetic additions to phosphopantothenate are then released by intracellular enzymes (esterases) and the resulting phosphopantothenate bypasses PANK and is converted to CoA. This cellular pathway was established by showing that intact dual-labeled ([^{18}O]phospho[^{13}C]pantothenate) fosmetpantotenate was converted to CoA.[7] Fosmetpantotenate raised liver CoA levels in mice, but an elevation of brain CoA could not be demonstrated.[7] However, the intrastriatal injection of fosmetpantotenate elevated brain CoA,[6] confirming the low to absent BBB penetration when fosmetpantotenate was delivered systemically. Although fosmetpantotenate is a promising bypass drug, inefficient BBB penetration is a significant liability.

Phosphopantetheine is another intermediate in the CoA biosynthetic pathway downstream of PANK, and phosphopantetheine itself[8] or its derivative, acetyl-phosphopantetheine,[9] was proposed as another bypass option. There are 2 serious problems with these potential therapeutics. (1) Both compounds are phosphorylated and, like phosphopantothenate, do not diffuse across cell membranes. The isotopic labeling experiment that was used to demonstrate the incorporation of the intact phosphopantetheine into cellular CoA cannot rule out the possibility that it was first degraded to pantetheine or pantothenate and then phosphorylated by PANK prior to incorporation into CoA.[8] Pantetheine is an excellent substrate for the PANK enzyme.[10] Because pantetheine is readily degraded to pantothenate by cultured cells and in circulation,[11] degradation prior to incorporation is the likely route to CoA. (2) The HoPan-treated mouse model[12] was used to demonstrate that acetyl-phosphopantetheine reversed the effect of HoPan on liver CoA levels in mice.[9] Because pantothenate alone potently counteracts HoPan-mediated reduction of CoA levels[12] and acetyl-phosphopantetheine is degraded to pantothenate by digestion,[13-15] it is not proven that the therapy bypasses PANK. The HoPan-treated mouse model is also not a representative PKAN model because HoPan treatment can be used to reduce CoA in the liver and kidney, but HoPan does not reduce brain CoA.[12]

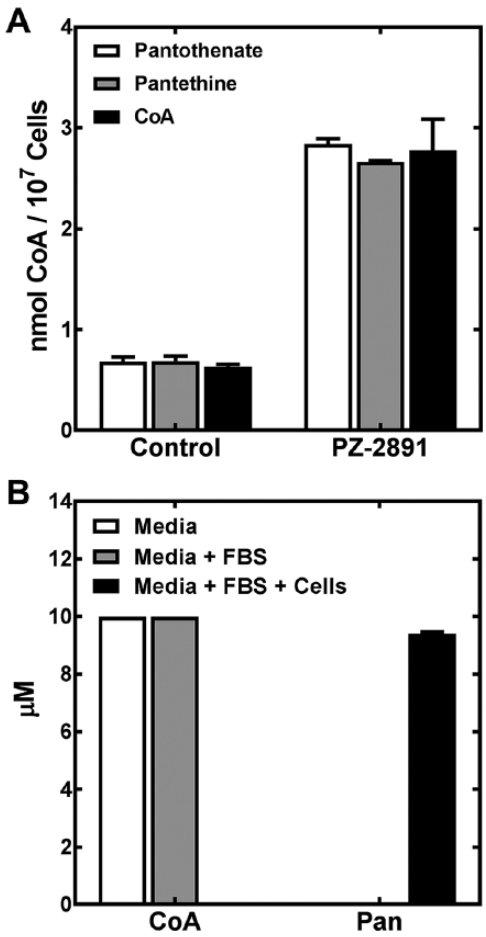

Figure 1. Elevation of cellular CoA and stability of CoA in cell culture. (A) C3A cells were treated with 10 µM pantothenate, pantetheine, or CoA in the presence or absence of PZ-2891 (10 µM). Total CoA was measured as described.[7] Neither extracellular CoA nor pantetheine elevated intracellular CoA. (B) CoA (10 µM) was incubated with DMEM culture medium, medium plus 10% fetal bovine serum (FBS), or medium plus serum plus C3A cells for 20 hours. The medium was analyzed by mass spectrometry for CoA and pantothenate.[5] CoA was stable in culture medium plus serum, but was quantitatively degraded to pantothenate when C3A cells were present. CoA indicates coenzyme A; DMEM, Dulbecco's Modified Eagle Medium.

Extracellular CoA itself was the third proposed bypass treatment using cultured neuronal cells derived from PKAN patient fibroblasts[16] or *Drosophila*.[8] The facts that highly charged molecules such as CoA[15] or CoA biosynthetic precursors[7] cannot diffuse into cells and that CoA is digested by an assortment of extracellular enzymes to ultimately yield pantothenate and cysteamine prior to absorption by intestine or other tissues[13,14] were not considered. There is no direct evidence that the treatment of cells with CoA increases cellular CoA.[8,16] Extracellular CoA, pantetheine, or pantothenate is not effective in raising CoA levels in human cultured cells (Figure 1A). CoA and pantetheine are completely degraded to pantothenate during cell culture (Figure 1B).

The BBB penetration challenge was addressed by the recent development of a novel drug called PZ-2891.[5] The physicochemical properties of drugs capable of crossing the BBB are established and PZ-2891 was designed to have the properties of polar surface area, number of hydrogen bond donors, molecular weight, and a cLogP value similar to the top 25 CNS drugs (Table 1).[17] By comparison, the properties of fosmetpantotenate provide a clear rationale for the difficulties encountered in elevating brain CoA with this therapy (Table 1). PZ-2891 is very lipophilic and diffuses across membranes to elevate cellular CoA levels by acting as an allosteric activator that prevents feedback inhibition of the PANK enzymes,[5] including PANK1 and PANK3 which are intact activities in the context of mutated PANK2 as found in PKAN patients.

A PKAN mouse model with disrupted brain CoA biosynthesis was derived by specific deletion of the murine *PANK1* and *PANK2* genes in neurons to serve as a platform to evaluate PKAN therapeutics.[5] Brain CoA levels were reduced significantly in this model and the animals exhibited phenotypic characteristics that resembled PKAN including reduced locomotor activity, growth rate, and lifespan. Oral administration of PZ-2891 elevated brain CoA and substantially resolved the severe locomotor, growth, and lifespan phenotypes.[5] These data show that PZ-2891 penetrates the BBB to elevate

Table 1. Physicochemical properties of PZ-2891, protected phosphopantothenate (RE-024 or fosmetpantotenate), and the top 25 central nervous (CNS) system drugs.

PROPERTY	MEAN VALUE OF TOP 25 CNS DRUGS	SUGGESTED LIMITS	PREFERRED RANGE	PZ-2891	RE-024
PSA (Å)	47	<90	<70	72	149
HBD	0.8	<3	0-1	0	3
cLogP	2.8	2-5	2-4	2.3	0.42
MW	293	<500	<450	349	474

Abbreviations: HBD, hydrogen bond donor; MW, molecular weight; PSA, polar surface area.
Properties of the top 25 drugs considered critical for blood-brain barrier penetration were taken directly from Hitchcock and Pennington.[17] The properties of PZ-2891[5] and RE-024, also known as fosmetpantotenate,[6,7] are provided for comparison.

CoA in CoA-deficient neurons to ameliorate the severe consequences of the CoA deficiency. There remain many challenges in the development of PKAN therapeutics, but the identification of a class of small molecule allosteric PANK activators that efficiently cross the BBB is an important step toward clinical deployment of a safe and effective treatment.

Acknowledgements

The author thanks the members of the St Jude Pantazine team, particularly Charles O Rock, Richard E Lee, and Stephen W White, as well as Lalit K Sharma, Chitra Subramanian, Mi-Kyung Yun, Matthew W Frank, Rajendra Tangallapally, Anne Edwards, Julie Maier, Katie Creed, Karen Miller, Jina Wang, Lois Richmond, and Ruobing Zhou for their hard work and support during the project.

Author Contributions

SJ conceived and wrote the article and data were provided by the St Jude Pantazine team.

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