Commentary

Insights into deoxyribonucleoside therapy for mitochondrial TK2 deficient mtDNA depletion

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Mitochondria are intracellular autonomous organelles with a separate genome (mtDNA) and translation system. They are involved in numerous cellular pathways, however their main function is to generate ATP from nutrients, by oxidative phosphorylation (OXPHOS) via the mitochondrial respiratory chain (MRC) composed of ~90 proteins organized in five multimeric complexes (CI-CV). CI-CIV transfer electrons originating from the tricarboxylic acid cycle to oxygen, forming water, while the proton gradient formed by CI, CIII, CIV is utilized by CV to generate ATP. Only 13 MRC proteins are encoded in the mtDNA (CII is solely nuclear encoded) while the remaining mitochondrial proteins including those required for the replication and translation of the mtDNA are nuclear encoded.

Mitochondrial diseases affecting OXPHOS is a prevalent but heterogeneous group of disorders (manifesting as infantile encephalomyopathy to adult onset blindness), transmitted either by maternal or Mendelian inheritance. A subgroup, “mtDNA depletion syndromes” or “mtDNA maintenance defects”, initially identified by the group of Mo-raes, Schon and DiMauro [5], is characterized by failure to maintain mtDNA synthesis and integrity, albeit a normal mtDNA sequence. The underlying molecular mechanism began to unravel almost a decade later, when the Hirano group identified mutations in thymidine phosphorylase (the enzyme responsible for the breakdown of deoxythymidine (dT)), in patients with neuro-gastro-intestinal-encephalo-myopathy (MNGIE), while coining the term defects of mtDNA maintenance defects (dNTPs) is crucial for maintaining mtDNA integrity, [7], and that the variable manifestations could be attributed to tissue specific distribution of the enzymes involved [10]. These findings also led to the hypothesis, that restoration of the imbalanced mitochondrial dNTP pools could be therapeutic for some of these devastating disorders.

Two research groups, mainly based in the US and Spain, decided to explore this option, in TK2 deficiency, a specific disease leading to reduced mitochondrial dTTP and dCTP pools. After proof of concept in mouse models [4], and subsequent to discussions by an eminent consortium of clinicians and researchers at a European Neuromuscular Centre workshop meeting (organized by Ramon Martí and Michio Hirano https://www.enmc.org/download/recommendations-for-treatment-of-mitochondrial-dna-maintenance-disorders/), the safety and efficacy of deoxyribonucleoside monophosphate (dNMP) and deoxyribonucleoside (dN) therapies were evaluated in TK2-deficient myopathy patients. The recently published results were generally positive, as clinical benefits in patients with early onset were notable, while patients with late onset profited less from the therapy [2].

Published in this issue of EBioMedicine, two teams, a Spanish-Swedish (Blázquez-Bermejo and colleagues) and US-based (López-Gómez and colleagues), present further evaluations of the efficacy and response to dN therapy, while addressing several key issues such as bioavailability, tissue specificity, age-related changes, and mode of administration.

The Spanish-Swedish group used an encephalopathy phenotype TK2 knockout model, to demonstrate that administration of dT + dC and dT alone, prolonged life expectancy and rescued mtDNA copy number in skeletal muscle. Bioavailability was studied by showing elevated plasma levels of dNs, but only in young mice, while older mice showed significantly lower levels in plasma and in tissues. In the absence of TK2, phosphorylation of administered dT and dC depends on the catalytic activity of cytosolic dN kinases, therefore the researcher’s also measured the expression and activities of the two relevant enzymes, TK1 and dCK, and found reductions in older animals. Additionally, they studied the phosphatases responsible for dNT degradation which could influence their bioavailability, and preliminary results showed organ-specific differences [1].

The US group studied the mechanisms for the incomplete response to treatment, in another mouse model, the Tk2 knock-in, and showed that indeed the levels of Tk1 and dCK in target tissues are critical for

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the efficacy of dN treatment. They also showed that parenteral administration more efficiently elevated dT, dC and mtDNA levels, than oral administration. Nevertheless, treatment had no significant effect in the brain or prolonged survival. Additionally they investigated the expression of TK1 and dCK in human muscle and observed decreased expression in adult compared to infant tissues [3].

Taken together, these two studies provide additional insights into dN therapy in mitochondrial TK2 deficiency. The age-related findings could actually explain the lower efficacy of dN administration in late onset TK2 deficiency [2]. The present results and future studies, will have practical implications by providing bench-to-bedside guidance for patient treatments in TK2 deficiency.

It should be emphasized that dN therapy, for other mtDNA maintenance disorders, must be meticulously investigated, depending on the specific defect, prior to administration to patients, bearing in mind that MNGIE is actually caused by an excess of dT and that maintaining correctly balanced dNTP pools is critical.

Declaration of Competing Interest

The author declares no conflicts of interest.

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