KL1333; NAD⁺ Modulator for Mitochondrial Diseases

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Abstract

Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is the most common maternally inherited mitochondrial disease due to A3243G mutation resulting in the mitochondrial dysfunction through the defect of the mitochondrial respiratory chain assembly. The increase of intracellular ROS levels and decrease of ATP levels are often found in MELAS patients. There are some treatment options for MELAS such as nutrients, antioxidant and vitamins, but curative treatment is not available.

Here, we investigated the effects of a novel NAD⁺ modulator KL1333 in MELAS. KL1333 reacted with NADP/oxidoreductase (NQO1) as a substrate, resulted in an increase of intracellular NAD⁺/NADH ratio. The increased NAD⁺ levels induced by KL1333 triggered the activation of two metabolic sensors SIRT1 and AMP-activated protein kinase (AMPK), and then subsequently activated peroxisome proliferator-activated receptor gamma coactivator (PGC-1α), a master regulator of mitochondrial biogenesis and function.

To examine the efficacy of KL1333 in MELAS, we used human fibroblasts derived from patient with MELAS harboring the A3243G mutation. Cells were treated with KL1333, and then the changes of intracellular ATP, lactate and ROS levels were measured. As a result, KL1333 significantly induced an increase of ATP levels, and decrease of lactate and ROS levels. In addition, KL1333 showed stronger effect on ATP levels than that of idebenone. In mitochondrial functional analysis, KL1333 increased the mitochondrial membrane potential, mitochondrial mass, and oxygen consumption rate (OCR).

Taken together, these results suggest that KL1333 can be a very promising therapy for MELAS (and also for other mitochondrial diseases) by increasing intracellular energy levels, antioxidant capacity and mitochondrial function through the reaction with NQO1.

Intracellular NAD⁺/NADH ratio

Results (1)

Figure 1: KL1333 increases NQO1 activity and intracellular NAD⁺/NADH ratio. (A) Compounds (each 5 μM) were used to measure NQO1 enzyme activity. (B) Human cells were treated with 2.5 μM KL1333 for indicated times. (C) Compounds (each 5 μM) were used to measure NQO1 enzyme activity. (D) Human cells were treated with 2.5 μM KL1333 for indicated times.

AMPK and SIRT1 in vivo and in vitro

Figure 2: KL1333 activates AMPK and SIRT1 in vivo and in vitro. (A & B) C2C12 myoblasts were treated with 1 μM KL1333 for indicated times. (C & D) db/db mice were sacrificed after 15 weeks of 80 mg/kg KL1333 daily oral administration. AMPK and SIRT1 activity in liver are shown.

PGC-1α induction

Figure 3: KL1333 induces PGC-1α activation. (A) & (B) C2C12 myoblasts were transfected with Flag-rPGC-1α for 48 hours, and then treated with 1 μM KL1333 for indicated times. The acetylation and phosphorylation levels of PGC-1α were measured by immunoprecipitation assay. (C) C2C12 myoblasts were transfected with rPGC-1α for 24 hours, and then treated with 1 μM KL1333 for 24 hours. PGC-1α titer was measured by Western blot analysis.

ATP levels

Figure 4: KL1333 increases ATP levels and decreases lactate and ROS levels in human fibroblasts derived from patient with MELAS. Human fibroblasts were treated with 1 μM KL1333 for 24 hours, and then intracellular ATP (A), lactate (B) and ROS (C) levels were measured by each assay. Human fibroblasts were obtained from Gangnam Severance Hospital.

Conclusion

KL1333 can be a very effective therapy for MELAS (and also for other MRCD [mitochondrial respiratory chain disease]) by increasing intracellular energy levels, antioxidant capacity and mitochondrial function through the reaction with NQO1.