Inhibition of electron transport chain at complex I improves glucose metabolism through decreasing $NAD^+/NADH$ ratio

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Background and aims: Accumulating evidences showed metformin and berberine, well-known glucose-lowering agents, were able to activate adenosine monophosphate-activated protein kinase (AMPK). Strikingly, they were also found to be able to inhibit mitochondrial electron transport chain at complex I. Whether AMPK or complex I is the target of metformin and berberine remains in conflict. In the present study, we aimed to explore the hypoglycemic effect of complex I inhibition and illuminate the role of AMPK in the beneficial effects of complex I inhibition.

Methods: Rotenone, amobarbital and gene silence of NDUFA13 were used to inhibit complex I. Intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT) were performed in db/db mice. Lactate release and glucose consumption were measured to investigate glucose metabolism in HepG2 hepatocytes and C2C12 myotubes. Glucose output was measured in primary hepatocytes. Compound C and recombinant adenoviruses expressing dominant negative forms of AMPK α 1 (D159A) and AMPK α 2 (K45R) were exploited to inactivate AMPK pathway. Western blotting was conducted to analyze protein expression. Cellular $NAD^+/NADH$ ratio was assayed to evaluate energy transforming and redox state.

Results: Rotenone ameliorated hyperglycemia and insulin resistance in db/db mice (Figure 1). In HepG2 cells, at concentrations between 0.02 and 0.2 $\mu\text{mol/L}$, rotenone improved glucose consumption by 20.1% to 31.7% and lactate production by 30.2% to 56.1% in a dose-dependent manner. Similar effects were observed in C2C12 cells. In primary hepatocytes, rotenone significantly inhibited glucose output by 30.6% to 72.4% in a dose-dependent manner (Figure 2). Rotenone also activated AMP-activated protein kinase (AMPK). Furthermore, it remained effective with AMPK inactivation (Figure 3). The enhanced glycolysis and repressed gluconeogenesis correlated with a reduction in cellular $NAD^+/NADH$ ratio, which resulted from complex I suppression (Figure 4). Amobarbital, another representative complex I inhibitor, stimulated glucose consumption and decreased hepatic glucose output in vitro, too. Similar changes were observed while expression of NDUFA13, a subunit of complex I, was knockdown with gene silencing.

Conclusions: These findings reveal mitochondrial complex I emerges as a key drug target for diabetes treatment. Inhibition of complex I improves glucose homeostasis through reducing cellular $NAD^+/NADH$ ratio in an AMPK-independent way.

FIG. 1. Rotenone improved glucose homeostasis and insulin sensitivity in db/db mice. (A) fasting blood glucose levels, (B) food intake, (C) body weight, (D) fasting serum insulin concentrations, (E) HOMA-IR and QUICKI, (F) OGTT and (H) ITT curves, (G and I) AUC of OGTT and ITT from rotenone or vehicle treated mice. * $P < 0.05$, ** $P < 0.01$ vs. corresponding baseline. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. db/db mice treated with vehicle.

FIG. 2. Rotenone promoted glycolysis and inhibited gluconeogenesis without cytotoxicity in cells. After incubation with rotenone, lactate production, glucose consumption and LDH concentration were measured in (A, C and E) HepG2 hepatocytes and (B, D and F) C2C12 myotubes. (G) Glucose output and (H) LDH concentrations in primary hepatocytes were measured after rotenone treatment. Data are expressed as means $\pm$ SEM. (n=4-8); * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. corresponding control.

FIG. 3. Rotenone enhanced glycolysis in the absence of AMPK activation. Representative western blot for AMPK, ACC, phospho-AMPK α (Thr172) and phospho-ACC (Ser79) in HepG2 cells (A) and C2C12 cells (B) preincubated with compound C or DMSO. Lactate release and glucose consumption were determined in HepG2 (C and E) and C2C12 myotubes (D and F). Western blot for AMPK, ACC and phospho-ACC (Ser79) in HepG2 cells (G) and C2C12 cells (H) infected with adenoviruses expressing control GFP reporter protein or DN-AMPK. Lactate release and glucose consumption were determined in HepG2 (I and J) and C2C12 (J and L) cells. Data are expressed as means $\pm$ SEM (n=3-8). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. the cells without rotenone treatment; # $P < 0.05$, ## $P < 0.01$ vs. the cells without compound C treatment or DN-AMPK infection.

FIG. 4. Rotenone increased cellular NADH content, decreased cellular $NAD^+/NADH$ ratio and inhibited complex I-linked respiration. NADH levels, NAD^+ levels and $NAD^+/NADH$ ratios were determined in HepG2 hepatocytes (A, D and G), C2C12 myotubes (B, E and H) and primary hepatocytes (C, F and I) after rotenone treatment. (J) The liver mitochondrial oxygen consumption rate was measured after rotenone treatment. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control.