SUPPLEMENTAL INFORMATION

DMT efficiently inhibits hepatic gluconeogenesis involving Gαq signaling pathway

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This Supplemental Information Includes:

Supplemental materials and methods

References

Supplemental Figure Legend

Supplemental materials and methods

Modified HGP assay

Modified HGP assay was conducted according to the previous study (Rah and Kim 2015). In the assay, primary hepatocytes were seeded onto a 48-well plate, and incubated with corresponding compounds in serum-free minimum essential medium (MEM) for 16 h. After washing with PBS twice to remove the remaining glucose, cells were incubated with compounds and glucagon (100 nM) in 200 μl glucose production detection buffer (glucose-free DMEM without phenol red containing 20 mM sodium lactate and 2 mM sodium pyruvate). After 5-h incubation, 50 μl detection buffer was collected for glucose concentration measurement with a colorimetric glucose assay kit (Nanjing Jiancheng, Nanjing, China) according to the manufacturer's instruction. The results were normalized to the total protein concentration measured by BCA protein kit (Thermo Scientific, MA, USA).

Preliminary pharmacokinetic assay

18～20 g-weight male C57BL/6 mice (n=3) were used for pharmacokinetic study. In the assay, mice were treated with DMT (10 mg/kg) by intraperitoneal injection, and then detected the concentrations of DMT in plasma at 0.08, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72 and 168 h.

Lactate content assay

Lactate content assay was performed as previously described (Qiu, et al. 2010). Primary

hepatocytes were seeded onto a 24-well plate and incubated overnight. The hepatocytes were treated with DMT and positive control (metformin) in serum-free MEM for 4 h. Lactate in the medium or serum of *db/db* mice treated with DMT for 5 weeks was measured with a lactate assay kit (Nanjing Jiancheng, Nanjing, China) according to the manufacturer's instruction.

References

Qiu BY, Turner N, Li YY, Gu M, Huang MW, Wu F, Pang T, Nan FJ, Ye JM, Li JY, et al. 2010 High-Throughput Assay for Modulators of Mitochondrial Membrane Potential Identifies a Novel Compound With Beneficial Effects on db/db Mice. *Diabetes* **59** 256-265.

Rah SY & Kim UH 2015 CD38-mediated Ca^{2+} signaling contributes to glucagon-induced hepatic gluconeogenesis. *Scientific Reports* **5**.

Supplemental Figure Legend

Supplemental Figure 1. DMT enhances AKT phosphorylation without increasing lactate production

(**A**) Modified HGP assay was performed according to the published approach (Rah and Kim 2015). Primary hepatocytes were incubated with corresponding compounds in serum-free MEM for 16 h. The cells were then incubated with compounds and glucagon (100 nM) in detection buffer. After 5-h incubation, 50 μl detection buffer was collected for glucose concentration measurement. (**B**) Primary hepatocytes were treated with DMT (20 μM) for 0.5, 1, 2, 4, 8 and 12 h, and then collected for western blot assay with antibodies against p-AKT and AKT. (**C**) Relative levels of p-AKT/AKT in (**B**) from three independent experiments. (**D**) 18～20 g-weight male C57BL/6 mice (n=3) were treated with DMT (10 mg/kg) by intraperitoneal injection. The concentrations of DMT in plasma were then detected at 0.08, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72 and 168 h. (**E**) The hepatocytes were treated with DMT and positive control (metformin) in serum-free MEM for 4 h. Lactate content in medium was measured with a lactate assay kit. (**F**) Lactate content in serum of *db/db* mice treated with DMT (25 mg/kg/day) for 5 weeks was measured with a lactate assay kit. All data were presented as means ± SEM (**p<0.01, ***p<0.001; ns, no significance).