Mitochondria-targeted ubiquinone (MitoQ) enhances acetaldehyde clearance by reversing alcohol-induced posttranslational modification of aldehyde dehydrogenase 2: A molecular mechanism of protection against alcoholic liver disease

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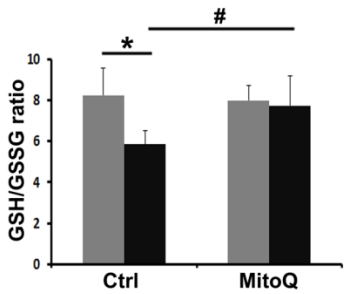
Supplementary Methods

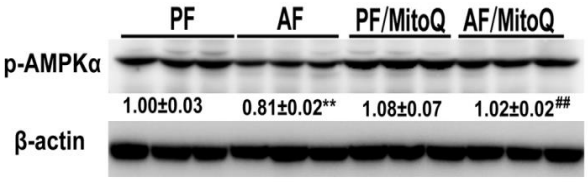
Biochemical analysis

The plasma ALT and AST activities were measured with an Infinity kits (Thermo Scientific, Waltham, MA). GSH and GSSG concentrations in the liver were determined by a commercial kit (BioVision, Milpitas, CA). The levels of triglycerides and free fatty acids in liver and plasma were measured with Triglyceride Assay kit (Thermo Scientific, Waltham, MA) and Free Fatty Acid Assay Kit (BioVision, Milpitas, CA), respectively, as described previously [1]. Aldehyde dehydrodenase (ALDH) activity was measured by a commercial Kit (BioVision, Milpitas, CA) followed by the manufacturer's instructions. Briefly, acetaldehyde was oxidized by ALDH in the homogenates of liver, ileum mucosa, or liver subcellular fractions. The generated nicotinamide adenine dinucleotide (NADH) then reduced a colorless probe to a colored product with strong absorbance at 450 nm. The ALDH activity was calculated as mmol NADH/min/mg protein. Serum H_2O_2 levels were measured with an Amplex Red Hydrogen peroxide/Peroxidase Assay kit (Invitrogen, Waltham, MA) as described previously [2]. The endotoxin levels in plasma were assessed by a chromogenic kinetic limulus amebocyte lysate assay kit (Lonza, Allendale, NJ) followed by the manufacturer's instructions. Liver contents of NAD^+ and NADH were determined by a commercial kit (BioVision, Milpitas, CA) as previously described [3]. Nitrite contents were measured by a Griess Reagent Kit (ACROS, NJ) followed by the manufacturer's instructions.

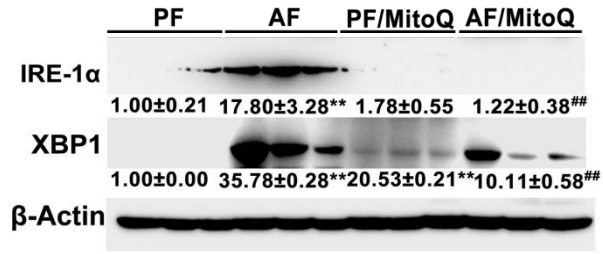
Supplemental Figures



Supplemental Fig. 1 MitoQ supplementation ameliorated chronic alcohol-decreased GSH/GSSG ratio. * P < 0.05 vs PF, # P<0.05 vs.AF.



Supplemental Fig. 2 MitoQ reversed alcohol-decreased hepatic p-AMPKα protein levels. * P < 0.05 vs PF, # P<0.05 vs.AF. ## P<0.01 vs.AF. Proteins levels were quantitated by NIH image J. All values are denoted as means ± SD



Supplemental Fig. 3 MitoQ reversed alcohol-increased hepatic IRE-1α and XBP-1 protein levels. * P < 0.05 vs PF, # P<0.05 vs.AF. ## P<0.01 vs.AF. Proteins levels were quantitated by NIH image J. All values are denoted as means ± SD

Supplemental Table 1

Name	Forward	Reverse
Mcp-1	GGAAAAATGGATCCACACCTTGC	TCTCTTCCTCCACCACCATGCAG
TNFα	TACTGAACTTCGGGGTGATTGGTCC	CAGCCTTGTCCCTTGAAGAGAACC
KC	GCGAATTCACCATGATCCCAGCCACCCG	GCTCTAGATTACTTGGGGACACCTTTTAG
Claudin-1	GTTTGCAGAGACCCCATCAC	AGAAGCCAGGATGAAACCCA
Occludin	CTCCCATCCGAGTTTCAGGT	GCTGTCGCCTAAGGAAAGAG
18s	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG

Supplementary References

[1] W. Zhang, W. Zhong, Q. Sun, X. Sun, Z. Zhou, Hepatic overproduction of 13-HODE due to ALOX15 upregulation contributes to alcohol-induced liver injury in mice, Scientific reports 7(1) (2017) 8976.
[2] Q. Sun, W. Zhang, W. Zhong, X. Sun, Z. Zhou, Pharmacological inhibition of NOX4 ameliorates alcohol-induced liver injury in mice through improving oxidative stress and mitochondrial function, Biochimica et biophysica acta 1861(1 Pt A) (2017) 2912-2921.
[3] Q. Li, G. Xie, W. Zhang, W. Zhong, X. Sun, X. Tan, X. Sun, W. Jia, Z. Zhou, Dietary nicotinic acid supplementation ameliorates chronic alcohol-induced fatty liver in rats, Alcoholism, clinical and experimental research 38(7) (2014) 1982-92.