Effects of amino acids on reversal of aminooxyacetate-induced metabolic disturbance

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Background: Malate-aspartate (MA) shuttle, locating on the inner membrane of mitochondria, is essential for maintaining the cellular bioenergetic states via the redox and transaminase reactions. Aspartate aminotransferase (AST) is required for the balances of the amino acids participating in the shuttle. In cytosolic compartment, NAD\textsuperscript{+} is formed to carry reducing equivalents into mitochondria. Therefore, the impairment of the MA shuttle would cause metabolic disturbance and dysfunction of mitochondria, leading to cell damage. Thus, we examine the effects of Aminooxyacetate (AOA), an aminotransferase inhibitor, on HepG2 cells; and investigate whether the treatment of amino acids would reverse the changes.

Methods: The activities of AST were analyzed to assess the inhibitive effects of AOA. Concentrations of metabolites including aspartate, glutamate, malate, α-ketoglutarate, citrulline, NAD\textsuperscript{+} and NADH were measured by a liquid chromatography tandem mass spectrometry (LC-MS/MS). The integrity of mitochondrial membrane potential (MMP) was evaluated by flow cytometry with Rhodamine dye.

Results: AOA inhibited AST activity in a dose-dependent manner (0.25-5.0 mM, p<0.0005 for trend analysis). In addition, 1 mM AOA treatment decreased intracellular aspartate (mean value 13.62 vs. 7.54 μmol/g, p<0.0005) and malate (21.99 vs. 5.37 μmol/g, p<0.0005) and increased glutamate (102.13 vs. 147.19 μmol/g, p<0.005). The ratio of aspartate to glutamate and malate to α-ketoglutarate in cells were decreased (0.14 vs. 0.05 and 3.22 vs. 0.53, respectively, p<0.0005). Furthermore, 1 mM AOA treatment increased the intracellular ratio of NADH to NAD\textsuperscript{+} (0.83 vs. 0.76, p<0.005) and caused the disruption of MMP (p<0.0005). Supplement of specific amino acids to AOA-administrated cells changed the concentrations of metabolites. The supplement of pyruvate reversed the elevation of NADH to NAD\textsuperscript{+} ratio. And glycine treatment protected cells from the disruption of MMP.

Conclusions: The treatment of pyruvate and glycine may protect cells against the AOA-induced cell damage.