Basis for Bicarbonate Damage in Myocardial Ischemia/Reperfusion Injury

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Bicarbonate is the main cellular buffer, acts as counter ion in carriers and possess an underappreciated redox activity that may contribute to injury. We observed that HL-1 cardiomyocytes exposed to increased [CO₂/HCO₃⁻] had more cell death after hypoxia/reoxygenation (H/R) and Langendorff-perfused rat hearts had larger infarcts after ischemia/reperfusion (I/R). In order to study the redox effects of high [CO₂/HCO₃⁻] during ischemic injury, we clamped the pH using HEPES and used the mouse cardiomyocyte HL-1 cell line and isolated perfused rat hearts. HL-1 cells exposed to 10% CO₂/HCO₃⁻ had no damage under basal conditions but developed exaggerated protein carbonylation and cell death after H/R. In Langendorff-perfused rat hearts, 10% CO₂ was well tolerated during baseline conditions but resulted in increased protein carbonylation, cell death and larger infarct areas after I/R. We hypothesized that the increased oxidative damage to proteins could be due to mitochondrial dysfunction with greater ROS production, diminished proteasomal degradation of oxidized proteins, or impaired autophagic clearance of damaged mitochondria and oxidized protein aggregates. There was no differential effect of CO₂ on mitochondrial morphology or proteasomal activity in HL-1 cells. In isolated heart mitochondria under low and high CO₂ conditions, there was no difference in ROS production or oxidized protein content, suggesting that increased CO₂ concentration does not worsen mitochondrial damage. Examination of autophagy in HL-1 cells exposed to high CO₂ during H/R revealed higher LC3-II and lower p62 content. In hearts, we detected accumulation of p62 in mitochondrial fraction, less mitochondria-associated Beclin1, and significantly more LC3 mRNA in hearts exposed to 10% CO₂ during I/R. In whole heart lisesates there was no difference in TOM70 (degraded by the proteasome), while there was accumulation of COX IV (degraded by autophagy) in the bicarbonate group. Taken together, these findings suggest that 10% CO₂ affects mitophagy, leading to the accumulation of oxidized proteins. These findings point to a protective role for autophagic clearance of oxidized protein aggregates during I/R injury that may be adversely impacted by bicarbonate therapy.

Key Words: Bicarbonate, ischemia reperfusion, heart