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MEETING ABSTRACT

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A novel role for adenosine kinase (ADK) in cardiac autophagy

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Background: Proper control of autophagy is important for cardiomyocyte homeostasis and adaptation to stress, as both excessive and insufficient autophagy have been implicated in heart failure development. LC3 is a ubiquitin-like (UBL) protein important for autophagosome formation and processing. Lipidation of LC3-I (conjugation to phosphatidylethanolamine; LC3-II) is vital for its role(s) in autophagy. LC3 lipidation is mediated by the sequential actions of an E1-activating enzyme, ATG7, an E2-conjugating enzyme, ATG3, and an E3-like ligase composed of ATG12–ATG5. While significant progress has been made in understanding how metabolic stress induces formation of autophagosomes, the physiological signals that restrain basal autophagy, particularly at the initial steps of LC3 activation and lipidation, are undefined. Adenosine exerts numerous protective effects in the cardiovascular system through stimulation of adenosine receptors, but its role in cardiac autophagy is not clear. The main route of myocardial adenosine removal is through intracellular phosphorylation and recycling into the adenine nucleotide pool by adenosine kinase (ADK). Here we used the ADK inhibitor, ABT-702, to investigate the role(s) of adenosine and ADK activity in cardiomyocyte autophagy.

Methods: To examine the *in vivo* role of ADK and adenosine signaling on cardiac autophagy, mice were injected i.p. with the ADK inhibitor, ABT-702 (10 mg/kg; 1–3 hrs) in the presence or absence of the adenosine receptor antagonist, theophylline (20 mg/kg). Body temperature was measured as an indication of globally increased interstitial adenosine. LC3-I, LC3-II, ATG7 (E1), ATG3 (E2), and ATG12–ATG5 (E3) proteins, as well as phospho-AMPK^{Thr172} and phospho-p70S6k^{Thr389} were measured by western blot. LC3 and ATG12 thioesters were examined by western blot under non-reducing conditions. For autophagic flux analysis, bafilomycin (3 µm/kg) was injected to inhibit lysosomal LC3 degradation. To examine the role of adenosine metabolism in cardiomyocyte LC3 lipidation and autophagic flux, cultured neonatal rat ventricular cardiomyocytes (NRVMs) were treated with adenosine (10 µM, 5 hrs) in the presence or absence of ABT-702 (0.3 µM) and/or bafilomycin (50 nM) and analyzed by western blot and immunofluorescence for LC3-II- and LC3-positive vesicles, respectively.

Results: ABT-702 increased cardiac LC3-II levels within 1 to 3 hours *in vivo*. The ABT-702-induced increase in LC3-II was not blocked by adenosine receptor antagonism with theophylline, indicating an adenosine receptor-independent mechanism. Co-administration of ABT-702 and bafilomycin further increased LC3-II formation, indicating that ADK inhibition increases LC3-II synthesis, rather than blocking its degradation. Conversely, treatment of NRVMs with adenosine inhibited LC3-II formation, and this effect was reversed by ABT-702 treatment, indicating that ADK metabolism of adenosine inhibits LC3 lipidation. *In vivo*, ABT-702 treatment increased formation of DTT-sensitive ATG12–ATG7 and LC3–ATG7 thioester complexes and

increased ATG12–ATG5 conjugation prior to greater increases in LC3-II, indicating that ABT-702 stimulates ATG7 activity.

Discussion: These findings indicate that ADK metabolism of adenosine restrains LC3 lipidation and autophagy in the heart. Thus, inhibition of ADK with ABT-702 may provide a novel approach for increasing autophagy in protein aggregate related diseases of the heart and possibly other organs.

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