Autophagy 7:4, 432-433; April 2011; © 2011 Landes Bioscience

Acute induction of autophagy as a novel strategy for cardioprotection

Getting to the heart of the matter

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Key words: myocardial ischemia, myocardial infarction, ischemia-reperfusion injury, autophagy, chloramphenicol

Abbreviations: CAPS, chloramphenicol succinate; LC3-II, microtubule-associated protein 1 light chain 3-II; MI, myocardial infarction; mTOR, mammalian target of rapamycin; PtdIns3K, phosphatidylinositol 3-kinase

Submitted: 11/26/10

Revised: 12/02/10

Accepted: 12/06/10

DOI: 10.4161/auto.7.4.14395

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Punctum to: Sala-Mercado JA, Wider J, Undyala VVR, Jahania S, Yoo W, Mentzer RM, et al. Profound cardioprotection with chloramphenicol succinate in the swine model of ischemia-reperfusion injury. Circulation 2010; 122:179–84; PMID: 20837911; DOI: 10.1161/CIRCULATIONAHA.109.928242.

There is no question that necrosis and apoptosis contribute to cardiomyocyte death in the setting of myocardial ischemia-reperfusion. Indeed, considerable effort and resources have been invested in the development of novel therapies aimed at attenuating necrotic and apoptotic cell death, with the ultimate goal of applying these strategies to reduce infarct size and improve outcome in patients suffering acute myocardial infarction (MI) or 'heart attack'. However, an issue that remains controversial is the role of autophagy in determining the fate of ischemic-reperfused cardiomyocytes: i.e., *is induction of autophagy detrimental or protective?* Recent data from our group obtained in the clinically relevant, in vivo swine model of acute MI provide novel evidence of a positive association between pharmacological upregulation of autophagy (achieved by administration of chloramphenicol succinate (CAPS)) and increased resistance to myocardial ischemia-reperfusion injury.

Ongoing controversy concerning the role of autophagy in myocardial ischemia-reperfusion injury (pro-survival versus pro-death) may be an example of the 'Goldilocks Principle': uncontrolled 'pathophysiological' induction of autophagy in response to an extreme or prolonged stress reportedly contributes to cardiac cell death, whereas a modest 'physiological' upregulation of autophagy may be beneficial. Indeed, in support of this latter concept, a growing body of evidence obtained in isolated cardiomyocytes and rodent models has revealed that acute, pre-ischemic induction of autophagy can confer a cardioprotective phenotype. The objective of our recent publication was to extend this paradigm to a clinically relevant, large animal (swine) model and establish whether pharmacological upregulation of autophagy would render the heart resistant to lethal ischemia-reperfusion injury and thus limit myocardial infarct size.

To test this concept, anesthetized pigs were assigned to receive our candidate drug, chloramphenicol succinate (CAPS) or placebo and, at 10 min after treatment, underwent 45 min of coronary artery occlusion followed by 3 h of reperfusion. Administration of CAPS results in a rapid and robust upregulation in molecular markers of autophagy: at 10 min post-treatment (the time corresponding to the onset of the sustained ischemic insult), we observed a 2.4- and 6.2-fold increase in expression of Beclin 1 and LC3-II, respectively, versus baseline. However, most notably, CAPS-treated pigs displayed a profound, ~50% reduction in infarct size when compared with placebo-controls. To investigate whether the favorable effect of CAPS was retained when administered in a more clinically relevant manner, an additional cohort of pigs received CAPS at 15 min before the onset of reperfusion. Efficacy was maintained (albeit attenuated) with delayed treatment, with mean infarct size reduced by ~27% versus controls.

The novel aspect of our study is the unequivocal documentation of a

significant infarct-sparing effect of CAPS in a well-established pre-clinical model of ischemia-reperfusion injury, thereby bringing the concept of cardioprotection via pharmacological upregulation of autophagy one step closer to future clinical evaluation. Nonetheless, our use of the swine model has an inherent weakness: although we have shown compelling evidence of an *association* between induction of autophagy and reduction of infarct size, the pig is not amenable to the application of genetic and molecular tools that would yield definitive documentation of *cause-and-effect*.

As acknowledged in our recent publication, an issue of particular relevance in establishing the mechanism by which CAPS confers cardioprotection is the tight and complex interaction between autophagy and the PtdIns3K-Akt-mTOR signaling pathway. Specifically, class III PtdIns3K is an activator of autophagy and, via its interaction with Beclin 1, plays a pivotal role in initiating autophagosome formation, whereas class I PtdIns3K purportedly suppresses autophagy. Interaction at the level of mTOR is multifaceted, bi-directional and has been reported to exert both positive and negative feedback; i.e., while activation of mTOR is associated with inhibition of autophagy, there is evidence of self-regulation of autophagy by autophagy-induced inhibition of mTOR and, in at least one model, co-activation of autophagy and mTOR. PtdIns3K-Akt-mTOR are also components of the 'Reperfusion Injury Salvage Kinase' or RISK pathway, a canonical cardioprotective signal transduction pathway that, when activated, has been shown in multiple models to attenuate lethal ischemia-reperfusion injury. Akt signaling is upregulated by a host of protective strategies including ischemic preconditioning (considered the 'gold standard' of cardioprotection) and pharmacological preconditioning-mimetic agents. It is therefore perhaps not surprising that administration of CAPS is accompanied by an increase in expression of phospho-Akt.

If CAPS treatment is associated with both an induction of autophagy (as documented in our study) and, as with many cardioprotective strategies, upregulation of Akt signaling, this raises two intriguing and interrelated possibilities. First, autophagy and PtdIns3K-Akt-mTOR signaling may yield additive benefit. Second, we speculate that co-activation of the Akt signaling pathway may, by these complex bi-directional interactions, assist in establishing an appropriate balance and maintaining autophagy in a favorable, pro-survival 'Goldilocks' state. Our data clearly demonstrate that CAPS is cardioprotective, and may, via induction of autophagy, provide a novel and clinically relevant therapy to attenuate myocardial ischemia-reperfusion injury. However, detailed molecular investigation will be required to 'get to the heart' of the mechanisms underlying the reduction of infarct size seen with CAPS treatment.