Each year 17 million people die from cardiovascular diseases, mainly acute myocardial infarction (AMI) and stroke. AMI is a disabling disease leading to heart failure and death. More specifically, coronary heart disease is the most common cause of death in the U.S., in Europe and in industrialized countries causing the death of approximately one in five men and one in six women. Infarct size is a major determinant of myocardial functional recovery and mortality after acute myocardial infarction (AMI) [1]. Limitation of infarct size is required to prevent post-ischemic heart failure and improve survival. Prompt revascularization of AMI by reperfusion (using thrombolysis or primary coronary angioplasty) leads to improved functional myocardial recovery and increased patient survival dramatically [2, 3].

Unfortunately, the reestablishment of blood circulation providing oxygen to hypoxic tissues activates apoptotic cascades that culminate in death of cardiac cells that were viable before reperfusion [4, 5]. Ischemia-reperfusion injuries contribute to about 50% to the final infarct size. Since no pharmacological agent has proven efficient so far, interrupting apoptotic cascades with peptides has been considered as a potential strategy to prevent reperfusion injuries. The BH4 (for Bcl2-homology) domain is shared by several pro-apoptotic proteins and index of anti-apoptotic activity. Furthermore, similar tools could be used to limit ischemic injuries in other organs and situations.

Cardioprotective properties of Tat-BH4 and Pip2b-BH4 in vivo

The BH4 peptide was appended to the Tat CPP was documented in cardiac cells and in vectors such as cell penetrating peptides (CPPs) has therefore been in vitro [6]. Anti-apoptotic properties have been demonstrated in several in vitro models. However, relatively large hydrophilic entities as peptides do not cross biological barriers. Their association with delivery vectors such as cell penetrating peptides (CPPs) has therefore been proposed to improve bioavailability.

The anti-apoptotic activity of constructs in which the BH4 peptide was appended to the Tat CPP was documented in cardiac cells and in the Langendorff reperfusion heart model but no in vivo evaluation has yet been published. Since new CPPs with seemingly improved ability to deliver biomolecules in muscle cells have been described recently, several such CPP-BH4 constructs were initially evaluated in primary cardiomyocytes. None was found more efficient than Tat-BH4 in terms of anti-apoptotic activity thus confirming that the CPP vector needs to be optimized for each transported cargo. The two most efficient constructs, Tat-BH4 and Pip2b-BH4, were then evaluated in vivo in a murine model of ischemia/reperfusion (I/R) [6]. In brief, ischemia was induced by a ligature of the left coronary artery and reperfusion of the myocardium was obtained by losing the knot. Anti-apoptotic peptide constructs or control ones were injected in the caudal vein. Remarkably, a single low dose (1 mg/kg) of either Tat-BH4 or Pip2b-BH4 reduces infarct size by about 50% when injected at the time of reperfusion. None of the controls, e.g., the CPP alone or scrambled versions of the CPP-BH4 constructs, had a significant anti-apoptotic activity. Maximal cardioprotection was reached when CPP-BH4 constructs were injected immediately before reperfusion that is just before the reperfusion-induced burst of apoptosis [7]. Moreover, injection 2 h after the beginning of reperfusion has no more cardioprotective effect. Parallel experiments did show that cardioprotection was accompanied by a reduction of DNA fragmentation, an index of anti-apoptotic activity.

Additional data concerning long-term benefits and toxicity as well as further investigations in other animal models (e.g., pig) will be required to forward the development of therapeutic tools to prevent reperfusion injuries after AMI. Likewise studies currently in progress in the group aim at a better understanding of the mechanism of cardioprotection and of the trafficking of these constructs in vitro and in vivo. Screening for other cardioprotective peptides or for tissue-specific delivery vectors is continued by the same group. The most unique thing about the cardioprotection by the optimized CPP-peptide constructs is that reduction in infarct size by 50% was achieved by only a low single dose injection at the time of reperfusion. While this may only be the first step demonstrating the possibility of a new treatment, its impact upon successful development into clinical practice will be certainly enormous.

Cover story

References


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