ATP-LOADED LIPOSOMES AND IMMUNOLIPOSOMES PROTECT ISCHEMIC MYOCARDIUM IN ISOLATED RAT HEARTS AND IN RABBITS WITH EXPERIMENTAL MYOCARDIAL INFARCTION

VLADIMIR P. TORCHILIN, D.D. VERMA, T.S. LEVCHENKO, W.C. HARTNER and E.A. BERNSTEIN
Department of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115, USA

In myocardial ischemia, a continuous absence of the oxygen supply leads to the energy failure and the loss of the ion balance of the cells. Exogenous ATP supply may protect myocardium from ischemic injury by improving recovery of mechanical function of myocytes. The purpose of this study was to develop a delivery system for ATP, a bioenergetic supplement, to the myocardium for protecting its mechanical function during the ischemia-reperfusion in the isolated rat heart model. Various methods of ATP encapsulation into liposomes have been investigated, and the freezing-thawing method has been chosen as the one providing the maximum ATP incorporation and not requiring the use of organic solvents. ATP-loaded liposomes (ATP-L) were infused during 1 min duration before starting the global ischemia for 25 min followed by reperfusion for 30 min in isolated rat hearts. The parameters providing information on the systolic and diastolic functions of the heart, left ventricular developed pressure (LVDP) and left ventricular end diastolic pressure (LVEDP) were recorded during the whole experiment. ATP-L provided a substantial protection to ischemic myocardium as compared to controls, free ATP (F-ATP) or empty liposomes (EL). The LVDP at the end of reperfusion in the ATP-L group significantly recovered to 72% of the baseline (good preservation of the systolic function) as compared to 26% of baseline in the group treated with the Kreb’s-Henseleit (KH) buffer, 40% of the baseline in the group treated with EL, and 51% of the baseline in the group treated with F-ATP. ATP-L-treated group showed also a significantly lower LVEDP (better preservation of the diastolic function) than the controls after the ischemia. At the end of the reperfusion, LVEDP significantly reduced to 23±3 mmHg in ATP-L group as compared to 59±6 mmHg in the KH buffer, 43±6 mmHg in the EL, and 31±2 mmHg in the F-ATP controls. After incubating the F-ATP and ATP-L with ATPase, the protective effect of the F-ATP was completely eliminated, while the protective effect of the ATP-L remained unchanged, i.e. liposomes should protect the incorporated ATP against fast enzymatic degradation in vivo.

The protective effect of ATP-liposomes can be still further improved if liposomes are modified with the monoclonal anti-myosin antibody 2G4 and, thus, acquire the ability to specifically recognize the ischemically damaged cells with compromised plasmic membranes. To prepare an ischemic myocyte-specific delivery system for ATP, ATP-containing immunoliposomes specific
for cardiac myosin were obtained by the chemical attachment of the 2G4 antibody to their surface. The ability of various preparations to protect an ischemic myocardium was again investigated in a Langendorff-instrumented, isovolumic rat heart model by measuring the LVDP and LVEDP during the experiment. After 25 min of total ischemia and 30 min reperfusion, the LVDP recovery was significantly better (up to 85% of the baseline) and LVEDP significantly reduced in the ATP-immunoliposome group, compared to all controls (free ATP, free liposome, ATP-liposome, and pure buffer groups).

ATP-loaded liposomes were also used as a bioenergetic supplement for the \textit{in vivo} protection of the myocardium in rabbits with experimental myocardial infarction. New Zealand White rabbits (2.5 – 3.5 kg) were anesthetized, intubated via a tracheostomy and ventilated. After exposing the heart through a parasternal thoracotomy, a flexible plastic catheter was inserted into the left atrium for rapid infusions. Left coronary artery was isolated and snared with a 3-0 suture for control of flow. Approx. 3 ml of ATP-L (45mg lipid/12mg ATP), or EL (45 mg lipids), or KH buffer, pH 7.4, were infused during a brief period of aortic occlusion and the occluding snare tightened. After 30 min, the snare was released and reperfusion established. After 3 hrs the coronary artery was re-ocluded, and 3 ml diluted Unispearse dye was infused via the atrial catheter to demarcate the area at risk. The anesthetized animal was immediately sacrificed. The heart was removed left ventricle sliced transversely between apex and base into approximately 5 equal slices, and digitally photographed on both sides to determine the area at risk. Slices were stained with nitroblue tetrazolium in PBS, pH 7.4 at 45°C for 20 min to detect the infarcted portion of the area at risk, re-photographed and weighed. The area at risk and the percent area at risk infarcted were determined from planimetry of both sides using Adobe Photoshop 7.0. The area at risk infarcted in the ATP-liposome group was significantly reduced (ca. 30% of the total area at risk) as compared to control EL- or KH buffer-treated rabbits (ca. 65% of the total area at risk) (p<0.05).

Thus, plain liposomes and anti-cardiac myosin immunoliposomes loaded with ATP effectively protect the myocardium by supporting its systolic and diastolic functions during ischemia and reperfusion both in vitro (isolated heart model) and in vivo (rabbits with experimental myocardial infarction). These results can be considered as a step towards the protection of the ischemic myocardium against damages resulting from the inadequate ATP supply.