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ATP-loaded liposomes effectively protect mechanical functions of the myocardium from global ischemia in an isolated rat heart model

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Abstract

ATP-loaded liposomes (ATP-L) infused into Langendorff-instrumented isolated rat hearts protect the mechanical functions of the myocardium during ischemia/reperfusion. The left ventricular developed pressure (LVDP) at the end of the reperfusion in the ATP-L group recovered to 72% of the baseline (preservation of the systolic function) compared to 26%, 40%, and 51% in the groups treated with Krebs–Henseleit (KH) buffer, empty liposomes (EL), and free ATP (F-ATP), respectively. The ATP-L-treated group also showed a significantly lower left ventricular end diastolic pressure (LVEDP; better preservation of the diastolic function) after ischemia/reperfusion than controls. After incubating the F-ATP and ATP-L with ATPase, the protective effect of the F-ATP was completely eliminated because of ATP degradation, while the protective effect of the ATP-L remained unchanged. Fluorescence microscopy confirmed the accumulation of liposomes in ischemic areas, and the net ATP in the ischemic heart increased with ATP-L. Our results suggest that ATP-L can effectively protect myocardium from ischemic/reperfusion damage.

Keywords: Isolated rat heart; Rabbit myocardial infarction; Mechanical functions; Ischemia; Reperfusion; ATP; Liposomes

1. Introduction

ATP levels drop by as much as 80% after 15 min in the cardiomyocytes during cardiac ischemia [1]. A similar time scale for the lowering of the intracellular ATP was observed during cardiac hypoxia and inhibition of glycolysis [2]. The concentration of ATP in the extracellular space in a well-perfused tissue is ~40 nM, [3] or ~10^5 times lower than the intracellular ATP concentration of 5–7 mM. In the continuing absence of the oxygen supply, both normal ATP supply and the

Abbreviations: ATP, adenosine-5′-triphosphate; ATPase, adenosine-5′-triphosphatase; ATP-L, liposomes loaded with ATP; F-ATP, free ATP in Krebs–Henseleit buffer; EL, empty liposomes; CPP, coronary perfusion pressure; LV, left ventricle; LVDP, left ventricular developed pressure; LVEDP, left ventricular end diastolic pressure; LVSP, left ventricular systolic pressure; KH, Krebs–Henseleit; EPR, enhanced permeability and retention; Rh–PE, rhodamine–phosphatidylethanolamine; FITC-dextran, fluorescein-isothiocyanate-labeled dextran; PEG, poly(ethylene glycol).

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temporary pathway for ATP production via glycolysis become rapidly depleted, ATP-dependent ion pumps in myocytes cease to function, cells lose their ion balance, swell, burst, and release their contents into the circulation. The mechanisms for the ischemia-induced myocardial failure include the depletion of ATP to 1.5–2.0 μmol/g wet weight and increase of intracellular Ca^{+} resulting in the increased myocardial contracture and cell death [4] by apoptosis or oncosis depending on duration of the ischemic stress and availability of the intracellular ATP [5].

The administration of the exogenous ATP might help in the restoration of its normal level in myocytes and cardioprotection. However, ATP has a very short half-life in the blood being hydrolyzed into ADP, AMP, and adenosine by extracellular ecto-nucleotidases [6]. Additionally, ATP is a hydrophilic and strongly charged anion, and cannot enter cells through the plasma membrane [6,7]. These limitations confine the direct use of the exogenous ATP as a therapeutic substrate. Thus, in order to generate a meaningful pharmacological response, alternative ways to deliver physiologically relevant amount of ATP into ischemic cardiomyocytes have to be found.

Liposomes are widely used as nanosized drug delivery vehicles [8]. The accumulation of liposomes as well as other nanoparticular drug carriers in the regions of experimental myocardial infarction was demonstrated [9–13], which proceeds via the enhanced permeability and retention (EPR) effect [14,15]. Liposomes may also "plug" and "seal" the damaged myocyte membranes and protect cells against ischemic and reperfusion injury in vitro [16]. Thus, one can suggest that ATP-L can be used for the "passive" ATP delivery into the infarcted myocardium.

Some encouraging results with ATP-L in certain in vitro and in vivo models have been reported. ATP-L protected human endothelial cells from the energy failure in a cell culture model of sepsis [17]. In a brain ischemia model, ATP-L increased the number of ischemic episodes tolerated before brain electrical silence and death [7,18,19]. In a hypovolemic shock-reperfusion model in rats, ATP-L provided effective protection to the liver [20]. ATP-L improved rat liver energy state and metabolism during cold storage preservation [21,22]. Biodistribution studies with the ATP-L demonstrated their accumulation in the damaged myocardium [23]. Several methods to load ATP into liposomes have been described, such as the reverse phase evaporation and emulsification [7,19,20,23]. We have recently shown that the freezing–thawing method provides a high degree of ATP incorporation into liposomes [24].

Here, we present the data on the cardioprotective effect of the ATP-L on isolated rat hearts subjected to global ischemia in the Langendorff isolated heart model, which is a reliable system for measuring systolic (the ability of the heart to contract) and diastolic function (the ability of the heart to relax) of the left ventricle (LV) as indexes of cardiac function after global ischemia and reperfusion [25]. These results can be considered as a significant step towards the protection of the ischemic myocardium against damages resulting from an inadequate ATP supply.

2. Materials and methods

2.1. Materials

Egg phosphatidylcholine (PC), cholesterol (Ch), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] ($mPEG_{2000}$-DSPE), 1,2-dioleoyl-3-trimethyl-ammonium-propane (DOTAP), and rhodamine–phosphatidylethanolamine (Rh–PE) were purchased from Avanti Polar Lipids (Alabaster, AL). ATP and adenosine-5′-triphosphatase (EC 3.6.1.3) from dog kidney (ATPase) were purchased from Sigma (St. Louis, MO). Fluorescein-isothiocyanate-labeled dextran (FITC-dextran) was purchased from Fluka Chemical (Buchs, Switzerland). All other chemicals and buffer components were of analytical grade.

2.2. Preparation of liposomes

To prepare ATP-L by the freezing–thawing method [24,26], chloroform solution of phosphatidylcholine (0.26 mmol), cholesterol (0.12 mmol), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethyleneglycol)-2000] ($mPEG_{2000}$-DSPE) (1.8 μmol), and 1,2-dioleoyl-3-trimethyl-ammonium-propane (10.8 μmol) was evaporated, and the film formed was hydrated with 5 mL of 400 mM ATP in the KH buffer. The dispersion was frozen at −80