Extending Hypothermic Storage Limits of Sensitive Primary Cell Systems

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Abstract

Cardiac transplantation exerts hypoxic, ischemic, and reperfusion stresses at the organ and cell level which must be considered during the preservation of sensitive primary cardiomyocytes. In an attempt to address these issues, several differing hypothermic solutions have been developed (1, 9). One main difference between the various types of preservation solutions lies in their proposed mechanisms of cardiac protection, either an extra-cellular or intracellular-like solutions. Intracellular-type solutions typically have been used in cold storage to limit damaging effects (1, 9).

Despite extensive research and solution development, successful heart preservation when coupled with hypothermic conditions to reduce physiological activity, remains limited to under 12 hours (3). The ability to store cardiac systems at hypothermic temperatures for extended periods would provide new avenues for transplantation and regenerative medicine in cardiac science. To understand the biocompatibility of these differing solutions, we have isolated adult and neonatal cardiac myocytes as models for hypothermic storage, ischemic conditions, and reperfusion injury. (2, 4-6 9, 10).

Introduction

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Methods

Cardiac myocytes were isolated from male Sprague-Dawley rats according to the Worthington Biochemical Neonatal Cardiomyocyte Isolation Kit (Worthington Biochemical, Lakewood, NJ) substituting SigmaBlend H collagenase (Sigma Chemical Co., St. Louis, MO).

Preservation of myocytes must address functionality as well as the general state of the cell. In this study, we examined the membrane integrity, metabolic activity, contractile function and cell viability of sensitive primary cardiomyocytes seeded on cell culture substrates and subjected to 24 or 48 hour hypothermic storage in either non-neuroprotective media or neuroprotective preservation solutions. These data should provide insights into the types of solutions useful for cardiac preservation and begin to address the importance of low temperature storage to limit damaging effects (1, 9).

Results

The use of an intracellular type storage solution, Hypothermolyn (HTS), was shown to be more sensitive to storage conditions than another intracellular type preservation solution, which exhibited more variable results (1, 9). Micrographs demonstrate the preservation of the cardiac cell monolayer, including cardiomyocytes and other supporting mesenchymal cells in the HTS series (24 hour storage) and in HTS-DCC and HTS-FRS (24 or 48 hour storage).

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Discussion and Conclusions

These data demonstrate differential survival of NRVM when cold stored for 24 to 72 hours in various preservation solutions and the importance of measuring preservation success by multiple methods. Establishing survival rates, metabolic activity, functional capability and protein expression provide supporting data to more accurately quantify the overall state of the cell following hypothermic preservation. Hypothermic preservation solutions which are formulated to contain free radical damage or chelate divalent cations (HTS-FRS and HTS-DCC) provided protection from both storage and as a result of hypothermic storage. Cells stored in these solutions maintained adherence to culture dishes and exhibited positive viability.

The survival ratio (Bcl-XL to Bcl-xL protein levels) drops below controls in all conditioned media cooled stored cells, but those conditions which do not afford protection demonstrate a more pronounced drop to below 50% of control samples. This may implicate the initiation of apoptotic cascades resulting from increased cell damage during the storage and recovery periods.

Regenerative medicine/cardiac therapies and transplantation science face a hierarchy of transport and storage of sensitive biologic products. The ability to preserve myocytes (survival and function) and supporting cellular matrix may be useful in advancing these fields over preservation hurdles.

References


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