Long-Term Protective Effects of Single-Dose Cardioplegic Solutions in Cell Culture Models

Gunaydin S, Akbay E, Gunertem OE, McCusker K, Onur MA, Ozisik K.

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ABSTRACT

Despite the popularity of single-dose cardioplegic techniques, the time window and targeted population for successful reperfusion remain unclear. We tested currently available techniques based on cell viability and integrity to demonstrate long-term cardioprotection and clarify whether these solutions were performed on neonatal/adult endothelium and myocardium by examining different cell lines. Cell viability with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test proliferation assay and membrane integrity with the lactic dehydrogenase (LDH) cytotoxicity test were documented in a cell culture/microscopy setting on adult (human umbilical vein endothelium [HUVEC]), neonatal (H9C2-cardiomyocytes), and myofibroblast (L929) cell lines. Apoptotic cell activity and necrosis were evaluated by acridine orange/propidium iodide (AO/PI) staining. Twenty-four hours after seeding, cells were incubated in control (Dulbecco's modified Eagle), St. Thomas and blood cardioplegia (4:1), histidine–tryptophan–ketoglutarate (HTK), and del Nido solutions at 32°C followed by an additional 6, 24, and 48 hours in standard conditions (37°C, 5% CO2). Experiments were repeated eight times. In MTT cell viability analysis, HTK protection was significantly better than the control medium in L929 cell lines at 48th hours follow-up and acted markedly better on the HUVEC cell line at 24th and 48th hours. del Nido and HTK provided significantly better protection on H9C2 (at 24th and 48th hours). Apoptotic and necrotic cell scoring as a result of AO/PI staining was found consistent with MTT results. The LDH test demonstrated that the level of cell disruption was significantly higher for St. Thomas and blood cardioplegia in H9c2 cells. Experimental studies on cardioplegia aimed at assessing myocardial protection use time-consuming and often expensive approaches that are unrealistic in clinical practice. We have focused on identifying the most effective cell types and the direct consequences of different cardioplegia solutions to document long-term effects that we believe are the most underestimated ones in the cardioplegia literature. Keywords: cardiopulmonary bypass, cardioplegia, cell biology/culture, myocardial protection, myocardial injury.

RESULTS

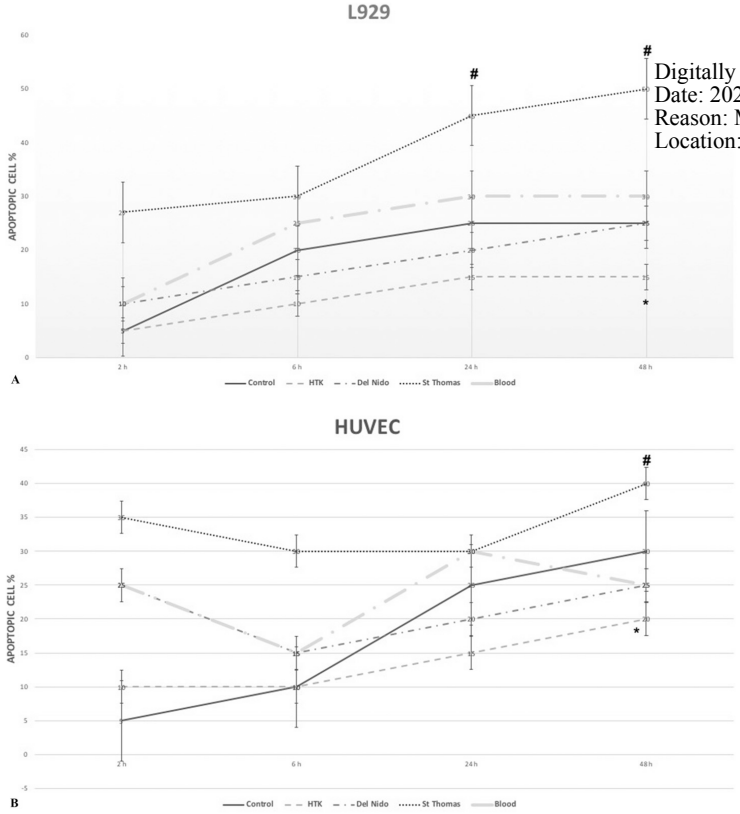

Figure 4. Apoptotic cell percentage of L929 (A), HUVEC (B), and H9C2 (C) cell lines after treatment with different cardioplegic solutions (*: $p < .05$ better with respect to control; #: $p < .05$, worse compared with control).

Apoptotic cell activity and necrosis:

a) St. Thomas significantly triggered late apoptosis and necrosis in all cell types

b) HTK and del Nido solutions caused significantly less cell death on neonatal/pediatric cells

c) HTK worked considerably well in late follow-ups with both pediatric and adult cardiac cell lines

CONCLUSIONS

I. Neonatal cardiomyocytes were significantly better protected with del Nido and HTK, and worked fine with the rather low 15–20% apoptosis/necrosis levels
II. Fibrous skeleton cells were significantly better protected by HTK than by the control medium
III. Adult endothelial cells showed better viability when protected with HTK in contrast to the control
IV. Late-term cell lysis by St. Thomas/blood cardioplegia may be due to the insufficiency of protection
V. Cytotoxicity did not differ significantly between groups