Preconditioning of cardiac tissue has proven to be a useful method of enhancing the heart’s ability to withstand stress experienced during ischemic periods typical of those encountered in heart surgery. Methods of preconditioning have included short exposure to elevated temperatures, ischemia, and various chemicals. Numerous studies have demonstrated the ability of brief periods of elevated temperatures to elicit protection against future lethal insults by inducing heat shock proteins (HSPs) [1-3]. Elevated levels of HSP 70 following thermal stimulation have been specifically linked to providing cardiac protection. Although the relationship between thermal stress and induction of HSP 70 role have been described there have been no description of the kinetics of heat shock protein expression as related to temperature and thermal stimulation time. We have investigated the relationship of HSP 70 level to thermal stimulation temperature and exposure time. With the ultimate goal being the design of a preconditioning treatment for cardiac tissue during surgery, HSP 70’s induction must occur over short time periods on the order of minutes or seconds. A study was conducted to determine whether higher temperatures coupled with shorter heating durations could produce elevated HSP70 levels while still maintaining high cell viability.

**MATERIALS AND METHODS**

A constant temperature circulating water bath was used as the thermal stimulation tool. Bovine aortic endothelial cells (BAECs) were the subjects of thermal stimulation since they are representative of cardiac tissue. BAECs were cultured to 90% confluence in culture flasks prior to the thermal insult. Heating medium preheated to the desired temperature was added and the flask was submerged in a constant temperature circulating water bath for time periods ranging from 1 to 30min. Three experiments were run with the water bath temperature at 46, 48, and 50 C with three samples for each time increment. After heating the flask was returned to the 37C incubator for 24 hours to allow the damage from heating to fully manifest itself. Following heating cell viability was assessed. Cells were stained with a two-component fluorescent dye and the percentage of live cells was determined by a flow cytometer. In order to predict and minimize cell injury in the future design of thermal preconditioning protocols related to temperature and exposure time, it was important to quantify cellular damage. Using the Arrhenius integral formulation for defining damage the percentage of viable cells was converted to numbers expressing damage. Linear regression of the damage vs. time curve yields the threshold time at each temperature and threshold temperature at each time. Extrapolating this curve to the logarithmic form of the Arrhenius equation, the critical kinetic coefficients for thermal damage, A and Ea, were determined. Determining these damage coefficients enables the thermal damage to be predicted for the BAEC’s cells by simply knowing the thermal fields generated by a heating source. The cells were lysed and underwent gel-electrophoresis and western blotting techniques to determine HSP 70 levels for each heating time and temperature. A HSP kinetics curve was generated for this data for all times and temperatures.

**RESULTS**

Based on the damage and HSP expression curves optimal times and temperatures were identified to maximize cell viability and HSP expression. From these studies it has been evident that a threshold temperature of 46C is sufficient to elicit a 10-fold increase in HSP 70 level compared to the control for exposure times longer than 2 minutes. Maximum HSP 70 induction occurs for thermal stimulation at 48C for a time of 7 minutes with a 44-fold increase and at 50C for a period of 2 minutes with a 30- fold increase compared to the control. For all temperatures and times ranging from 1-10 minutes cell viability remained above 90%. Values for damage for all temperatures and times between 1-10minutes ranged from 0-.08516.

**CONCLUSION**

Significant increases in HSP70 level can be achieved for thermal stimulation times on the order of minutes. Cell viability is also not compromised at these elevated temperatures for short durations. The damage information and HSP kinetics determined can be employed as design criteria for a thermal stimulation source such as...
ultrasound or microwaves to ultimately allow a thermal preconditioning protocol to be developed to enhance cardiac protection during surgery.

References

