Cryobiology 75 (2017) 125–133

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Determination of cryothermal injury thresholds in tissues impacted by cardiac cryoablation

Ryan P. Goff [a, b], Stephen G. Quallich [a, b], Robert A. Buechler [a, b], John C. Bischof [a, c, d], Paul A. Iaizzo [a, b, d, *]

[a] Department of Biomedical Engineering, University of Minnesota, 420 Delaware St. SE, B172 Mayo, Minneapolis, MN, 55455, USA
[b] Department of Surgery, University of Minnesota, 420 Delaware St. SE, B172 Mayo, Minneapolis, MN, 55455, USA
[c] Department of Mechanical Engineering, University of Minnesota, 420 Delaware St. SE, B172 Mayo, Minneapolis, MN, 55455, USA
[d] Institute for Engineering in Medicine, University of Minnesota, 420 Delaware St. SE, MMC 609, Minneapolis, MN, 55455, USA

ARTICLE INFO

Article history:
Received 23 August 2016
Received in revised form
29 December 2016
Accepted 3 January 2017
Available online 3 January 2017

Keywords:
Cryoablation
Phrenic nerve palsy
Cryothermal tolerance
Atrial fibrillation

ABSTRACT

Despite widespread clinical use of cryoablation, there remain questions regarding dosing and treatment times which may affect efficacy and collateral injury. Dosing and treatment times are directly related to the degree of cooling necessary for effective lesion formation. Human and swine atrial, ventricular, and lung tissues were ablated using two cryoablation systems with concurrent infrared thermography. Post freeze-thaw samples were cultured and stained to differentiate viable and non-viable tissue. Matlab code correlated viability staining to applied freeze-thaw thermal cycles, to determine injury thresholds. Tissue regions were classified as live, injured, or dead based upon staining intensity at the lesion margin. Injury begins at rates of ~10 °C/min to 0 °C, with non-viable tissue requiring cooling rates close to 100 °C/min to ~ −22 °C for swine and significantly greater cooling to −26 °C for human tissue ($p = 0.041$). At similar rates, lung tissue injury began at 0 °C, with human tissue requiring significantly less cooling, to ~ −15 °C for complete necrosis and −26 °C for swine ($p = 0.024$). Data suggest that there are no significant differences between swine and human myocardial response, but there may be differences between swine and human lung cryothermal tolerance.

1. Introduction

Cardiac balloon cryoablation for the treatment of atrial fibrillation has been gaining attention as an approach for isolation of the pulmonary veins since its first reported use in 2003 [2] and in the current clinical form in 2005 [33]. Focal cryoablation has been used surgically for many years [36], with transcatheter devices becoming available in more recent decades.

Despite widespread and growing clinical use, there are still questions regarding dosing and treatment times for cryoablation, which may affect both efficacy and collateral injury [11,12]. Parameters affecting RF ablation lesions are thoroughly characterized, and temperatures of 50 °C or higher are required for the creation of myocardial scars [19]. For instance, the effects of power, ablation duration, catheter orientation, catheter size, usage of irrigation, and contact forces on lesion size have been thoroughly studied for RF ablation [22,25,29,40]. This is particularly relevant given the release of a second generation cryoablation device with a different cooling profile that may affect operation [7]. The highest regularly detected clinical complication is phrenic nerve palsy, at rates of 4–8% [28,39]. One can appreciate how adjusting dosing may lead to better outcomes.

A survey of the literature was unable to uncover definitive data for hypothermic or cryoinjury reporting directly the temperatures of the myocardium and surrounding tissues impacted by ablation correlated directly to viability. This lack of data prompted the current study which, to the authors' knowledge, is the first to report these limits for cryoablation. However, much work has been performed on other tissues [16], cardiovascular tissue models [18], cells [37], and relating the dosing time to tissue outcomes [1,5], but lacking the direct tissue temperatures achieved. The novelty of the current study is direct tissue temperature measurements correlated to viability response.

Conversely, in the hyperthermic treatment area (i.e., RF, laser,

* Corresponding author. Department of Surgery, University of Minnesota, 420 Delaware St. SE, B172 Mayo, Minneapolis, MN, 55455, USA.
E-mail address: iaizz001@umn.edu (P.A. Iaizzo).

http://dx.doi.org/10.1016/j.cryobiol.2017.01.002
0011-2240/

etc.), there are data regarding injury to many tissues including the myocardium [23]. For instance, work by Pearce and colleagues [21,30] exploited cardiac tissue's unique, native birefringence to detect protein denaturation after thermal ablation. Importantly, cellular protein denaturation is thought to be one of the main mechanisms leading to necrosis from hyperthermic treatments. In Pearce's study, researchers measured the birefringence of collagen, an extracellular matrix protein, and correlated this to tissue destruction [30]. Unfortunately, a similar imaging technique cannot be easily implemented during or after freezing because protein denaturation is not a major mechanism of cryoinjury and has not been correlated with cellular injury after cryo therapy. More specifically, the mechanism of cellular cryoinjury is related to water phase change (i.e., intracellular ice crystal formation and cellular dehydration) during freezing [20,27] which has been studied in numerous cell types [3,4,9], although less in whole tissues [23,24]. Cooling below the actual freezing point of tissue is necessary for complete destruction, as shown in studies of the kidney [32,36] and liver [31] where cooling to −15 °C or below was required. Furthermore, cellular injury is known to be dependent on both end temperature and cooling rate, as described in reviews on cryosurgical injury in the literature [15,24].

It was therefore the goal of this work to determine what cooling regimes lead to necrosis, injury, or recovery (i.e., have no detectable effect on myocardium). The goal of the present study differs from experimentally reported catheter temperature effects on tissues [1,38] as the present work measures the tissue temperatures directly during the experiment. Infrared (IR) thermography was selected instead of a thermocouple, in order to measure temperature over a large area (i.e., every pixel produces a thermal profile). Additionally, IR is a noncontact measurement technique, which is particularly beneficial given the large thermal gradients produced during cryoablation (i.e., 100 °C/cm), and has been used previously for cryothermic [17] and hyperthermic [7] studies in cancer [17,34]. The drawback of IR thermography is that accuracy suffers in comparison to other techniques (i.e., ±2 °C, or 2% of total range), and that the system used is limited to detection down to −30 °C. This should be sufficient since tissue injury is expected below the phase change temperature of tissue (<−0.5 °C), down to a range of −15 or −20 °C based on previous work in liver and kidney [8,10,32]. To measure the correlative injury in cardiac tissue post cryoablation, histological and enzymatic staining were used as previously described [32].

2. Materials and methods

To benchmark this approach, swine kidney cortex (n = 6, from two animals) was obtained and ablated per the protocol described below for comparison to existing literature. Kidney cortex is homogenous with a smooth, flat surface allowing ease of ablation, IR monitoring, and post cryoablation TTC (triphenyltetrazolium chloride) staining. After reproducing results from the literature with kidney, the approach was then used on cardiac tissue including myocardium and a smaller set of lung samples.

2.1. Sample preparation

Female Yorkshire Cross swine hearts (n = 15 animals), lungs (n = 5, from 4 lungs), and kidneys (n = 6, dissected from 4 kidneys) were chilled and stored in modified Krebs-Henseleit buffer within 30 min post-mortem from the University of Minnesota Meat Sciences Laboratory (Minneapolis, MN, USA). Swine hearts were then transported to our laboratory within an hour. Human heart (n = 3) and lung (n = 3) specimens were obtained from non-viable cardiac transplant organ donors through the regional organ procurement organization, LifeSource (St. Paul, MN, USA) within 6 h of cross-clamp time. The human specimens were deemed non-viable by LifeSource due to not meeting standard transplantation criteria (e.g., unknown cardiac arrest time, cardiac comorbidities, etc.). The use of these human tissues was approved by the University of Minnesota IRB and LifeSource research committee. Human and swine samples were dissected from the atria and ventricles to a nominal thickness of 5 mm. Locations were selected that were relatively 'flat' with a low degree of invaginations to provide a uniform surface for IR imaging, as well as to avoid out-of-focus effects and non-symmetrical ablation lesions. Samples were placed in individual petri dishes with room temperature phosphate buffered saline.

The prepared samples were then placed in the apparatus and imaged by an IR camera, as shown in Fig. 1A. The apparatus consisted of a plastic petri dish with central ablation probe and 2 mm of Sylgard (Dow Corning, Midland, MI, USA) polymer formed to the bottom. This apparatus was designed to accommodate two cryoablation probes with different cooling powers. The first was the Galil Medical SeedNet system (Arden Hills, MN, USA) designed for prostate cryoablation, using an argon refrigerant to achieve cooling rates and end temperatures of approximately 400 °C/min and −120 °C at the probe surface in air. The second system was the Medtronic Cryocath system (Minneapolis, MN, USA) using the FreezorMAX catheter with cooling rate and end temperatures of approximately 300 °C/min and −84 °C in the air. Two different probes were used with the goal of generating different cooling profiles. The study sought not to characterize performance of these probes, but simply used them as tools to apply cooling consistently to the tissues. The samples ablated using the Galil system were transected by the needle in the middle, whereas the samples ablated using the Medtronic system had a 2 mm biopsy punch hole placed in the center of the sample through which the catheter was advanced. This created a consistent interference fit, which obviated the need for contact force control, as contract force will impact ablation lesion formation. Using this technique approximated a situation that was easy to model numerically, removed the variability of catheter contact pressure, and created uniform axisymmetric lesions (Fig. 1B). The lesion edge occurred well away from the injury induced by the biopsy punch or transection and therefore did not affect results.

2.2. Infrared imaging and viability assessment

Prior to sample placement in the petri dish, ~5 ml of ultrasound gel was distributed uniformly about the bottom of the dish. After placement of the sample, another volume of ultrasound gel was added to surround the tissue. Ultrasound gel has been shown to have very similar thermal properties to myocardium [13]. By surrounding the sample with ultrasound gel, we thereby minimized variation in sample size and created a more reproducible ablation volume as the entire dish has similar thermal properties.

After sample placement, the emissivity and appropriate environmental parameters were input to the infrared software (ThermaCAM Researcher Pro 2.9, FLIR Systems, Inc., Boston, MA, USA). Emissivity was determined by comparison of IR temperature to a 0.040″ T-type thermocouple (5SRTC, Omega Engineering, Stamford, CT, USA) being read by a Fluke 51II thermometer (Everett, WA, USA). A T-type thermocouple with data logging was affixed to the active cooling metal portion of the cryoprobe with Kapton (3 M, St. Paul, MN, USA) tape for later computer modeling. Cryothermal applications (i.e., ablations) of desired durations were subsequently performed.

After ablation, samples were washed with phosphate buffered saline, incubated with 60 ml of cell culture media (DMEM/F12, 10%