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Abstract
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Measurement and Prediction of Thermal Behavior and Acute Assessment of Injury in a Pig Model of Renal Cryosurgery

FRANZ R. SCHMIDLIN, M.D.,1 CHRISTOPHER C. RUPP, M.D.,2 NATHAN E. HOFFMANN,3 JAMES E. COAD, M.D.,2 DAVID J. SWANLUND,3 JOHN C. HULBERT, M.D.,1 and JOHN C. BISCHOF, Ph.D.3

ABSTRACT

Purpose: To analyze in vivo end temperatures and histologic injury in a standardized cryo-iceball using a porcine kidney model in order to establish the threshold temperature for tissue ablation. To evaluate the ability to predict end temperatures using a thermal finite element model.

Materials and Methods: A single freeze/thaw cryolesion was created in five pig kidneys and the temperature history recorded. End temperature was calculated using a thermal finite element model. The threshold temperature for tissue injury was established by directly correlating end temperature and histologic injury.

Results: Reproducible geometry and temperature profiles of the cryo-iceball were found. End temperature could be accurately predicted through thermal modeling, and correlation with histologic injury revealed a threshold temperature of −16.1°C for complete tissue ablation.

Conclusion: Thermal modeling may accurately predict end temperature within a cryo-iceball. Provided threshold temperatures for tissue destruction are known, modeling may become a powerful tool in cryosurgery, improving the assessment of damage in normal and malignant tissue.

INTRODUCTION

RENAL CRYOTHERAPY is currently being investigated as a treatment for localized renal-cell carcinoma (RCC). Preliminary results confirm the safety of this technique and indicate successful tumor eradication, but far more follow-up data will be needed before conclusions can be drawn.1–5 During cryosurgery, the extent of tissue destruction may be monitored either by ultrasonography or temperature measurement. Indeed, ultrasonography was found to be an excellent tool, as the margin of the iceball can be recognized easily as a hyperechoic rim.6 However, the periphery of the cryo-iceball contains a transition zone with a blend of viable and dead cells.7,8 Temperature monitoring was shown to be a more accurate predictor of tissue destruction, but at the present time, temperature may not be deduced from ultrasound images. Thermosensors have therefore been proposed as an objective and reproducible means for monitoring the extent of complete tissue destruction during cryosurgery.8

In this study, we evaluated a new method to assess temperature and histologic injury during kidney cryosurgery. The motivation was to provide the surgeon with a tool to predict temperatures during cryosurgery in order to noninvasively assess the actual size of the lesion representing complete tissue destruction. For such an endeavor, our present knowledge of the temperature history as well as the histologic tissue response to end temperature during cryosurgery is incomplete. The objectives of this study therefore were twofold: first, to analyze the in vivo end temperature in a standardized cryo-iceball using a porcine kidney model and correlate the end temperature with tissue damage, thereby establishing the threshold temperatures for histologic injury; and second, to evaluate the ability to predict the end temperature within the cryolesion using thermal modeling with the finite element method.

MATERIALS AND METHODS

After approval from the Institutional Animal Care and Use Committee, five kidneys from five mongrel swine were used in...
this study. After intubation, an indwelling catheter was placed for cardiovascular monitoring. With the animal in the supine position, a midline abdominal incision was performed to gain access to the kidneys. The animals were sacrificed 1 to 2 hours after cryosurgery.

**Cryosurgical Technique**

A single freeze/thaw cryolesion was created using an argon gas-cooled Cryocare™ cryosurgical system (Endocare, Irvine, CA). The probe (3.4-mm outer diameter, 40-mm cooling length) was inserted to a depth of 15 mm into the centrolateral renal cortex, and freezing was undertaken for 15 minutes followed by a 10-minute passive thaw cycle. The iceball progression was monitored visually and by ultrasonography. The thermal history was recorded with three thermocouples placed parallel to the cryoprobe at a tissue depth of 10 mm (Fig. 1) at increasing distance (5/10/15 mm). A special jig was used to keep the thermocouples and the cryoprobe in position during the procedure. Thermocouple conduction error was established in vitro by performing temperature measurements in kidney tissue frozen in three different constant temperature baths (−78°C/−20°C/0°C).

**Thermal Modeling**

To estimate the end temperature within the cryo-iceball, a finite element model was used. An axi-symmetric approximation of the iceball geometry within the kidney was created using the Finite Element Heat Transfer software package (FEHT; F-Chart, Middleton, WI). The actual dimensions of the model describing the cryo-iceball were based on the zone of hemorrhagic infarction. The cryolesion was assumed to be axi-symmetric. The governing thermal equation that was solved over the tissue domain was the Pennes bioheat equation. For the two phases in the model, the governing equation is:

\[
\frac{1}{\rho C_p} \frac{\partial T}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{k T}{r} \frac{\partial T}{\partial r} \right) + \frac{1}{\partial z} \left( k \frac{\partial T}{\partial z} \right) + \hat{m} C_{pb}(T_a - T_f)
\]

where \( j = u \) or \( f \) for unfrozen regions, respectively. The thermal parameters include \( \rho \), \( C_p \), \( k \), \( T \), and \( \hat{m} \), which are the density, specific heat, thermal conductivity, temperature, and blood perfusion rate, respectively, of each phase in tissue. Also, \( C_{pb} \) is the specific heat of blood and \( T_a \) is the temperature of arterial blood.

Several assumptions were imposed to simplify the solution of the equation: (1) assuming steady-state behavior at the maximum extent of the iceball; (2) the blood flow \( \hat{m} \) (perfusion source term) was 0 in the frozen and 2 mL/mL per min in the unfrozen tissue; (3) temperature-dependent thermal properties for the frozen region were used as previously described; and (4) metabolic heat was neglected. The geometry was based on an axi-symmetric two-dimensional steady-state finite element model. The boundary conditions for the in vivo model were that the probe boundary was at \( T_p \) (−135°C), as measured during the experiments, the tissue–iceball boundary was at \( T_{ph} \) (the phase change temperature for isotonic solutions, −0.53°C), the kidney far from the iceball was 37°C, and finally, the exposed face of both the iceball and the kidney was receiving heat by natural convection (\( h = 10 \text{ W/m}^2\text{K} \)) from the ambient temperature around the kidney, set at 37°C. This model was solved for the steady-state profile of temperature within the tissue. The mesh of the model was designed to have a node at the exact radius and depth of the three thermocouples during cryosurgery to allow easy comparison.

**Histology and Threshold Temperature**

The kidneys were dissected, enabling quantitative assessment of the cryolesion around the cryoprobe. Before further histologic processing, the kidney specimens were submerged at 21°C for 48 hours in RPMI 1640 Medium (GIBCO/BRL, Gaithersburg, MD) enriched with 1% penicillin–streptomycin and 2% fetal bovine serum. This treatment allows uninjured cells to survive, while damaged cells progress through the histologic changes of irreversible injury. Tissue was then Formalin fixed (10%) and stained with hematoxylin and eosin for histologic examination. The radial extension of the cryolesion was measured (N = 5) at the level of the thermocouple measurements (10-mm tissue depth), as shown in Figure 1. The extent of the iceball, total and partial glomerular and tubular death, as well as vascular integrity (endothelial cells and vascular smooth muscle) were measured throughout the lesion assum-
ing 10% shrinking secondary to histologic processing. This figure was based on permanent tissue markings applied before histologic fixation. Complete tissue ablation (TA) was considered to have occurred in the presence of tubular and glomerular death. Glomerular death was defined as pyknosis of all glomerular cell nuclei (both endothelial and mesangial cells), along with capillary thrombosis and distention and hemorrhage into Bowman’s space. Tubular death was identified as a mixture of coagulative necrosis and apoptosis characterized by cellular outline preservation along with nuclear pyknosis and karyorrhexis. Tissue damage was correlated with temperature profiles (end temperature) in order to establish threshold temperatures for injury. For this purpose, the end temperature at locations between the temperature measurements were extrapolated using the finite element simulation.

RESULTS

No intraoperative complications occurred, with blood pressures remaining stable throughout the procedure in all cases. Only minimal bleeding was observed at the cryoprobe insertion site.

Geometry

The extension of the cryo-iceball correlated well with the zone of hemorrhagic infarction found immediately after tissue thawing (Fig. 1). Macroscopic evaluation revealed a reproducible iceball (N = 5) characterized by a hemiellipsoid, axisymmetric lesion. The mean radius at largest extension was 15 mm with an overall tissue depth of 22 mm. One or more papillae were occasionally involved in the hemorrhagic zone.

Temperature Measurements and Temperature Prediction

The cryoprobe cooled at ~200°C/min and reached a mean tip temperature of −135°C with −62°C (5 mm), −22°C (10 mm), and +3°C (15 mm) being found at the thermocouple sites (Fig. 2). Temperature measurements were highly reproducible for all thermocouple sites with standard deviations between 2° and 3°C. The mean thermocouple conduction error was 4°C at −78°C, 1°C at −20°C, and 0.5°C at 0°C. The end temperature within the cryo-iceball was calculated as shown in Figure 3. There was close correlation (±3°C) between the calculated and recorded end temperatures. Thermal isotherms (−20°C/−40°C/−60°C) were calculated and displayed in a two-dimensional configuration (Fig. 4).

Histology and Threshold Temperatures

Microscopically, the lesion was well demarcated with hemorrhage into the interstitial tissue surrounding the glomeruli and tubules throughout the cryo-iceball. The radial extension of the iceball at the level of temperature recording (10 mm) was 13.3 mm. Complete tissue ablation (epithelial-tubular and glomerular death) was found as far away as 10.44 mm with an adjacent transition zone presenting a mixture of dead and living cells; glomeruli proved to be slightly more resistant than tubules. Blood vessels in the center of the lesion experienced extensive (75%–90%) smooth muscle and endothelial damage, whereas vessels at the iceball periphery had only a little damage (<10%). The threshold temperature for complete tissue ablation was −16.1°C (see Fig. 3).

DISCUSSION

Renal cryosurgery is particularly attractive for the treatment of unifocal malignancies such as RCC as it is capable of creating an iceball of significant size that can easily be monitored.
visually and by ultrasonography. However, the absence of histologically confirmed tumor-free margins remains a significant concern from an oncologic point of view. To overcome this obstacle, freezing beyond the visible tumor margin has been performed in order to achieve complete cancer cell kill in the target zone.\(^5\) Temperature monitoring may be a more reliable and objective predictor of tissue damage during cryosurgery.\(^6,11\) In this study, we evaluated a new method to improve the assessment of tissue destruction using temperature prediction as a substitute for histologic examination. The goal is to predict the temperature, and thus the extent of damage, within the cryo-iceball using simulation with a thermal finite element model. However, to predict temperatures and tissue necrosis reliably within a cryolesion, the following requirements need to be satisfied: the temperature history within a cryo-iceball has to be reproducible, the threshold temperature for histologic injury needs to be clearly established, and finally, there has to be a valid method for thermal modeling.

In this study, the temperature profiles taken in all kidneys were found to be in close agreement and highly reproducible, with variations limited to between \(2^\circ\) and \(4^\circ\)C (see Fig. 2). Schulsinger and associates\(^11\) had found much larger temperature variations (\(10^\circ\)–\(12^\circ\)C) in a canine model, but the absence of a jig holding the cryo-probe and the thermocouples in place during the surgery may explain the difference. Thermocouple conduction errors may also modify the results of temperature monitoring. However, our results show that thermocouple conduction errors play a role only in the center of the iceball, where temperature gradients were prominent. In the area of interest (toward the edge of the cryolesion), errors were reduced to \(1^\circ\)–\(2^\circ\)C, and temperature measurements may therefore be considered sufficiently accurate.

In renal tissue, a 3.4-mm cryo-probe typically creates a radially symmetric lesion, as shown in Figure 1 and reported in other clinical and experimental studies.\(^12-14\) Both the near-symmetric geometry and the reproducible temperature history within the cryo-iceball make renal cryosurgery an ideal field for the application of thermal models. As shown in Figure 3, we were able to accurately (\(\pm 3^\circ\)C) predict the end temperature at the thermocouple sites.

The computational efforts remain simple and rely on PC-based software that may be easily integrated into an operating room. In fact, only the macroscopic assessment of the cryo-iceball based on the zone of hemorrhagic infarction and the recorded probe tip temperature were needed for this simulation. During cryosurgery, the required geometry may be assumed on the basis of perioperative ultrasound measurements of the iceball in a completely noninvasive manner. The results of such simulations may be finally displayed as cross-sections of the lesion (Fig. 4). Within the cryo-iceball, the isotherms for different target end temperatures may be calculated and visualized in a 2D model. Such information may be used for preoperative planning or indicate perioperatively if the treatment parameters have actually ablated tissue for a specific tumor size. In our opinion, at the present time, the thermal modeling could be integrated during cryosurgery as an adjunct to the present treatment monitoring by ultrasonography. Additional \textit{in vivo} temperature measurements taken at specific locations during the procedure may be useful to validate the calculations, thereby significantly increasing the accuracy and oncologic safety of renal cryosurgery in a minimally invasive manner. Further refinement of the thermal model; i.e., to a 3D set-up, could allow this method to be applied to more complex clinical situations.

One major limitation is that the simulations were done in a quasistatic context, and therefore, the simulations are limited to calculating end temperatures. However, this limitation is acceptable, as end temperature has been identified as a prime factor in cell death by several groups.\(^7-15\) Indeed, a study performed on rat prostate tumor cells indicates end temperature and hold time as of greater importance than the cooling and thaw rate for the prediction of tissue injury.\(^15\)

To use a thermal model for cryosurgical planning, the threshold temperature for tissue necrosis must be well established. In this study, end temperature and histologic damage were directly correlated within the same tissue slice, and a threshold temperature of \(-16.1^\circ\)C was found for complete tissue ablation (see Fig. 3). This temperature threshold is close to the values reported in the literature by Chosy and associates (\(-19.7^\circ\)C)\(^8\) and Campbell and coworkers (\(-20^\circ\)C)\(^9\). However, the values were established in normal kidneys, and according to Gage and Baust,\(^7\) much lower threshold temperatures may be expected in malignant tissue. From the available empirical survival results in cancer cells, those authors recommend an imposed end temperature for all cryotreated tissue below \(-60^\circ\)C to ensure cell death. However, a marked difference has been observed be-

FIG. 4. End temperature may be calculated for any location within cryo-iceball. Figure shows a 2D display of iceball and isotherms for different target temperatures (\(-60^\circ\)C/\(-40^\circ\)C/\(-20^\circ\)C).
tween survival of cells treated in vitro and in vivo. Indeed, additional tissue destruction has been observed as part of a host-mediated response to the injury, allowing temperatures higher than \(-60^\circ\text{C}\) to \(-80^\circ\text{C}\) for in vivo treatment.\(^9\) A better understanding of the role of such additional tissue-related injury mechanisms in renal cryosurgery is part of our ongoing research.

**CONCLUSION**

Renal cryosurgery creates a typical axi-symmetric cryo-ice-ball with a reproducible temperature history (end temperature). End temperature may be directly correlated with tissue damage and, provided the macroscopic extension of the iceball and the probe tip temperature are known, it may be accurately predicted at any location within the cryo-iceball by the means of a simple PC-based thermal simulation using the finite element method. Thermal modeling, together with visual and ultrasound monitoring, may become a powerful surgical tool in cryosurgery by improving the assessment of cryosurgical tissue damage in normal and malignant tissue. However, further research will be needed to determine the threshold temperatures needed for tumor destruction in human kidneys.

**REFERENCES**


Address reprint requests to:
Franz R. Schmidlin, M.D.
Dept. of Urologic Surgery
University of Minnesota
420 Delaware St. SE
Minneapolis, MN 55455