

Transcatheter Cryoablation Part I: Preclinical Experience

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Introduction

The concept of cooling to treat medical disorders dates back to the ancient Egyptian Edwin Smith Papyrus on surgical trauma, written between 3000 and 2500 B.C. Hypothermic therapy was recommended for abscesses that were “oily, like fluid under thy hand, [which] produce some clamminess of the surface.”¹ Modern forms of cryothermal tissue ablation have been used surgically for decades in numerous organ systems and for various pathologies. Unlike heat that destroys cells by coagulation and tissue necrosis with potential for thrombus formation and aneurysmal dilatation, cryoablation involves a distinct pathophysiological process. As such, it carries a unique safety and efficacy profile. While not novel as an energy modality, harnessing cryoenergy into a steerable transcatheter format represents a more recent landmark in the history of arrhythmia therapy.

In Part I of this two-part series, we will focus on the body of knowledge underlying the development of a transcatheter cryoablation system. Pertinent features related to biophysics and mechanisms of cryothermal tissue injury will be highlighted, key historical developments considered, and experience gained from cryosurgery with hand-held probes summarized. Preclinical studies with transcatheter cryoablation will be detailed, setting the framework for human applications. Part II of this series will review the current state of knowledge regarding clinical experience with transcatheter cryoablation.

Mechanisms of Injury

The objective of cryoablation is to freeze tissue in a discrete and focused fashion to destroy cells in a targeted area. Simplifying complex mechanisms of cellular injury, tissue damage involves freezing and thawing, hemorrhage and inflammation, replacement fibrosis, and apoptosis.²

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Hypothermia causes cardiomyocytes to become less fluid as metabolism slows, ion pumps lose transport capabilities, and intracellular pH becomes more acidic.³ These effects are entirely transient, provided that the duration of nonfreezing cooling temperatures does not exceed a few minutes. Indeed, the briefer the exposure to a hypothermic insult, the more rapidly cells recover. As a clinical correlate, this characteristic of cryoenergy permits functional assessment of putative ablation sites (i.e., cryomapping) without cellular destruction. In contrast, the hallmark of permanent tissue injury is ice formation. As cells are rapidly cooled to freezing temperatures, ice crystals form within the extracellular matrix and then intracellularly as well.⁴ The size of ice crystals and their density is dependent on proximity to the cryoenergy source, the local tissue temperature achieved, and the rate of freezing. While the crystals do not characteristically destroy cell membranes, they compress and deform nuclei and cytoplasmic components.^{5,6} Mitochondria are particularly sensitive to ice crystals and are the first structures to suffer irreversible damage.^{7–9} Upon completion of freezing, the tissue passively returns to body temperature, resulting in a “thawing effect.” This is an important component of cryoablation, as rewarming causes intracellular crystals to enlarge and fuse into larger masses that extend cellular destruction.^{3,4,10,11}

Hemorrhage¹² and inflammation⁶ characterize the second later phase of cryoablation.² In what has been termed a “solution effect,” water migrates out of myocardial cells to reestablish the osmotic equilibrium that was disturbed by ice crystals. In effect, this increases the intracellular solute concentration to a hyperosmotic state that may damage cell membranes.¹⁰ As the microcirculation is restored to previously frozen tissue, edema ensues. The fluid traverses damaged microvascular endothelial cells, resulting in ischemic necrosis. In the final phase of cryoinjury, replacement fibrosis and apoptosis of cells near the periphery of frozen tissue give rise to a mature lesion within weeks.¹³ Typically, these lesions are well circumscribed, with distinct borders, dense areas of fibrotic tissue, contraction band necrosis, and a conserved tissue matrix, including endothelial cell layers.¹⁴

Initial Cryoablation Systems

Cryosurgical devices cooled by liquid nitrogen were introduced in the early 1960s.¹⁵ This technology was extended to treat a wide spectrum

Table I.
Historical Landmarks in the Development of a Transvenous Cryoablation System

Year Reported	Authors	Contribution
1948	Hass GM	Described the production of myocardial lesions with cryoenergy
1963	Cooper IS	Described the first cryosurgical apparatus
1964	Lister JW et al.	Applied cryoenergy to conduction tissue
1977	Harrison L et al.	Performed cardiac cryosurgery with a hand-held probe
1991	Gillette PC et al.	Conducted an animal study with a transvenous cryocatheter
1998	Dubuc M et al.	Steerable cryocatheter with recording and pacing electrodes
2001	Dubuc M et al.	First clinical study with transcatheter cryoablation

of pathologies including dermatologic, prostatic, hepatic, gynecologic, ophthalmologic, neurosurgical, and oncologic disorders.^{3,16–18} Preceding these widespread applications, Hass¹⁹ and Taylor et al.²⁰ first described predictable controlled myocardial lesions with cryoenergy using carbon dioxide as a refrigerant. Initial descriptions of tissue characteristics remain valid today. Notably, lesions were described as homogeneous and sharply demarcated with preserved ultrastructural integrity. These attributes, with absence of aneurysmal dilation or rupture, were attributed to the remarkable resilience of collagen and fibroblasts to hypothermal injury.²¹

Table I summarizes key historical landmarks in the development of a transvenous cryoablation system for cardiac arrhythmias.^{15,19,21–25} It was in 1964 that Lister et al.²² first described the application of cryoenergy to the cardiac conduction tissue by suturing a 4-mm “U”-shaped silver tube near the bundle of His. This may be considered the origin of “cryomapping” as well. Sinus node function was inhibited by cooling with an alcohol and carbon dioxide mixture at -10°C to -20°C . At the atrioventricular (AV) node, PR interval prolongation occurred at -45°C and progressed to high-grade AV block. Normal AV conduction resumed almost instantaneously upon discontinuation of cooling.

Cardiac Cryosurgical Experience

Atrioventricular Nodal Ablation

In 1977, Harrison et al.²¹ introduced cryosurgery with hand-held bipolar electrode probes, first in 20 dogs with AV nodal ablation followed by three patients with refractory supraventricular tachycardia. Under cardiopulmonary bypass, complete but reversible AV block was achieved in all patients when the temperature of the nitrous oxide probe was lowered to 0°C at the His bundle site. Permanent complete AV block resulted when the temperature was further lowered to -60°C for 90 to 120 seconds and at

least two consecutive freeze/thaw cycles were delivered. Longer-term follow-up on a larger series was later reported, with AV block achieved successfully in 17 of 22 patients.²⁶ Additional studies reported similar results.^{27–29}

Approaches not requiring extracorporeal bypass were later devised.^{28,30,31} Bredikis²⁸ described a technique consisting of two atriotomy incisions; one for digital palpation and the second for the cryoprobe. Positioning of the cryoprobe was guided by recording electrodes, cryomapping, and/or pressure-induced AV block. Using this method, complete AV block was achieved in 85% of 34 patients²⁸ and 92% of 72 patients.³⁰ Louagie et al.³¹ proposed an alternative epicardial approach via the right coronary fossa.

Accessory Pathways

Gallagher and coworkers³² reported the first two cases of successful cryosurgical accessory pathway ablation in 1977. One pathway was concealed and paraseptal and the second manifest and left-sided. Several case series followed,^{33–38} with the largest reporting an epicardial approach in 105 consecutive patients with Wolff-Parkinson-White syndrome (74 left lateral, 23 paraseptal, and 11 right ventricular free wall).³⁶ The AV fat pad was mobilized and dissected and the accessory pathway exposed and cryoablated. All but one patient had acutely successful ablation. However, four required repeat interventions for what the authors believed were subendocardial pathways protected by warming effects of circulating blood. A different approach to ablation was described in a series of 21 patients.³⁴ Left-sided pathways were targeted by cryoprobes designed to enter the coronary sinus, obviating the need for extracorporeal bypass. Overall, 19 of 21 patients were successfully treated. Acute rupture of the coronary sinus occurred in two instances and required surgical ligation.

Ventricular Tachycardia

In 1978, Gallagher et al.³⁹ cryosurgically ablated a pharmacologically resistant ventricular tachycardia focus in the anterior right ventricular free wall with three 90-second applications at -60°C . A second case was reported the following year.⁴⁰ Cryosurgery has since become a recognized treatment for selected patients with refractory ventricular arrhythmias,^{16,27,41-44} often as an adjunct to more extensive surgery including aneurysmectomy, subendocardial resection, encircling endocardial ventriculotomy, coronary artery bypass grafting, and valvar replacement.² To date, no prospective studies have compared cryosurgical efficacy and safety to other treatment modalities. With cryosurgery alone, Caceres et al.⁴⁵ and others⁴⁶ reported 93% event-free follow-up in patients with refractory ventricular tachycardia. These results compare favorably to historical cohorts that used other surgical modalities for ventricular tachycardia.⁴⁶⁻⁴⁸

Other Arrhythmia Substrates

Surgical cryoablation has also been described for less common arrhythmias including nodoventricular tachycardia,⁴⁹ sinoatrial reentrant tachycardia,⁵⁰ ventricular disabling bigeminy,⁵¹ bidirectional bundle branch reentry tachycardia,⁵² and fetal malignant tachyarrhythmias.⁵³ It has also been used in AV nodal reentrant tachycardia and other arrhythmias with rapid AV conduction with the shared objective of slowing but preserving nodal conduction.⁵⁴⁻⁵⁶ Holman et al.⁵⁷ successfully eliminated dual AV nodal physiology in three dogs. Cox et al.⁵⁸ later applied hand-held cryoprobes to eight patients with drug-refractory AV nodal reentry tachycardia. All patients were successfully treated without requiring permanent pacing, although right bundle-branch block was induced in three cases.

Cryolesion Characteristics

Animal studies of cryosurgical ablation have characterized lesions and demonstrated that dimensions relate to temperature of the cryoprobe and myocardium, probe diameter in contact with cardiac tissue, exposure time, and number of freeze/thaw cycles.^{12,59-61} Longer duration of freezing and lower temperatures produce larger lesions, although a plateau is reached within five minutes.^{6,10} Double freeze/thaw cycles generate larger lesions than single applications of longer duration.^{62,63} Such parameters could be varied to produce predictable lesions.^{11,61} As illustrated in Figure 1, cardiac cryosurgery is still used today, although less commonly. Insights gained from the cryosurgical experience contributed invaluablely to

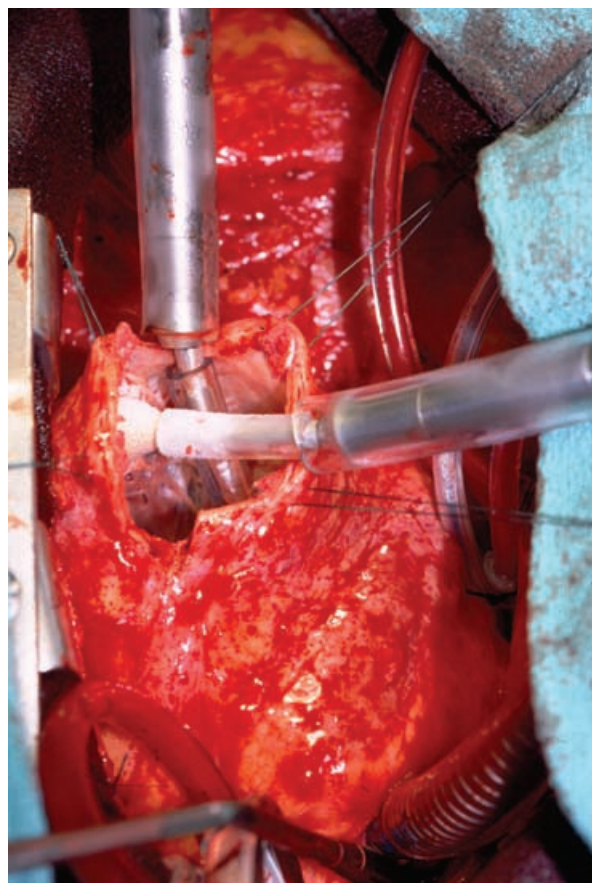


Figure 1. Surgical ablation with a cryoprobe.

conceptualizing the modern transcatheter cryoablation system.

Preclinical Studies with Transvenous Cryoablation

Original Transvenous Cryocatheters

Gillette et al. reported the first animal study using a transvenous cryocatheter in 1991.²³ In five miniature swine, complete AV block was produced with an 11-French cryocatheter cooled by pressurized nitrous oxide. Cryothermia was applied for three minutes and repeated up to three times. Four of the five pigs remained in AV block for one hour, while one recovered partially with 2:1 AV conduction. Histologically, acute lesions were sharply delineated and hemorrhagic. In a chronic study of eight swine, successive three-minute cryoapplications were delivered to the AV junction at -60°C via 8 or 11-French cryocatheters.⁶⁴ Long-term AV block was maintained in five of eight animals. At six weeks, well-defined dense lesions were noted histologically, free of inflammation or thrombus formation. Although feasibility of transcatheter cryolesion formation was demonstrated, limited success was

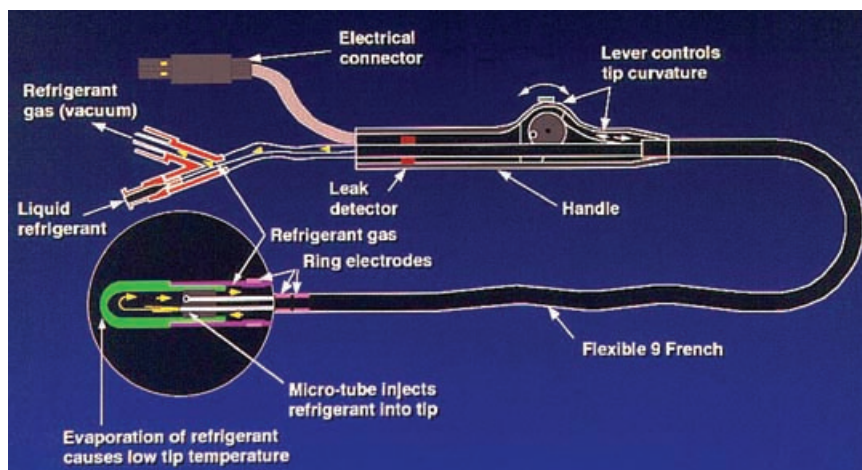


Figure 2. Catheter cryoablation system. Reproduced with permission from Dubuc et al.,²⁴ Please see text for a detailed description of the various components.

attributed to lack of steerability and recording electrodes. Cryocatheter placement required using a second catheter to record local signals.

Steerable Cryocatheter with Recording and Pacing Electrodes

Transcatheter cryoablation was revived several years later, ultimately leading to clinical use. We reported the first animal experiment using a steerable cryocatheter with integrated recording and pacing electrodes in 1998.²⁴ Right and left ventricular lesions were created in six dogs using a 9-French catheter with a 4-mm electrode tip and Halocarbon 502 (Freon®) as a refrigerant. Cryomapping (i.e., reversible ice mapping) of the AV node was demonstrated by sequentially applying lower temperatures to the AV nodal junction. When high-degree AV block or >50% PR prolongation was achieved, the cryoapplication was interrupted. In all cases, 1:1 AV conduction resumed within seconds. No lesion was identifiable on gross and microscopic histopathology. In a further study of cryomapping with more detailed electrophysiological measurements, reversible AV nodal effects were achieved in seven of eight dogs at a mean temperature of -40°C .¹³ Parameters including sinus cycle length, atrial-His (AH) interval, His ventricular (HV) interval, Wenckebach cycle length, and AV node effective refractory periods, measured before, 20 minutes, 60 minutes, and up to 56 days after cryomapping were not significantly different.

Chronic cryoablation lesions, created at a mean temperature -55°C , were later characterized in nine mongrel dogs sacrificed three and six weeks after ablation.¹³ Histologically, well-demarcated ultrastructurally intact lesions devoid of thrombus were observed. Similar results were

obtained with 8.5-French cryocatheters in six dogs⁶⁵ and seven pigs.⁶⁶

Optimal Freezing Parameters

To better define optimal cryoablation parameters, single versus double freeze/thaw cycles were compared at the lowest temperature (-50°C to -55°C) permitted by the system at the time.¹³ These lesions were applied to sites where cryomapping ($>-40^{\circ}\text{C}$) had been successful. Permanent chronic AV block was achieved in all six dogs with double freeze/thaw cycles compared to only one of six with single freeze/thaw cycles. Consonant with this observation, intralesion residual strands of viable tissue were noted histologically with single but not double freeze/thaw cycles. Thus, at these temperature and freezing rates, double cycles were more effective than single ones for AV nodal ablation. Larger lesions with more extensive tissue injury have been consistently reported with double freeze/thaw cycles applied to other organs as well.^{3,17} However, later iterations of the transcatheter cryoablation system permitted lower attainable temperatures (-80°C) and faster cooling rates when nitrous oxide was used as a refrigerant. Cryobiology experts have since refrained from systematically recommending double freeze/thaw cycles.

Preclinical studies contributed importantly to our understanding of the impact of cooling rate and catheter tip-temperature on tissue effects.^{3,13,16,24,27} Cooling first occurs at the distal catheter tip in contact with endocardial tissue. Freezing then extends radially into the tissues, establishing a temperature gradient. The lowest temperature and fastest freezing rate is generated at the point of contact, with slower tissue cooling rates

more peripherally. Of importance, as distant tissue achieves a temperature in the order of -20°C to -30°C , a “dynamic cryomap” is obtained. Reversible local tissue effects precede cell death. A clinical corollary is that despite an initial reassuring cryomap, vigilance for perinodal substrates is mandated as the iceball continues to expand during cryoablation and the centrifugal temperature gradient further extends into the tissue.^{12,13,24,60,61}

Transvenous Catheter Cryoablation System

The first cryosurgical device developed by Cooper in 1963¹⁵ produced cooling by means of a liquid to gas phase change in nitrogen. Principles such as the Joule-Thompson effect (cooling by expansion of a compressed gas after passage through a needle valve) and Peltier effect (thermoelectric cooling) have been incorporated into the design of cryoprobe.^{11,16} A variety of devices were developed using several methods of refrigeration and numerous cryogens including nitrogen, nitrous oxide, solid carbon dioxide, argon, and several fluorinated hydrocarbons.³

We initially described a transvenous cryocatheter system that used Halocarbon 502 (Freon[®]) as a refrigerant (Cryocath Technologies Inc., Montreal, Canada)²⁴ (Fig. 2). The refrigerant was later changed to Genetron[®] AZ-20⁶⁷ and then nitrous oxide,¹⁴ used currently. The cryocatheter essentially consists of a hollow shaft with a closed distal end containing a cooling electrode tip and three proximal ring electrodes for recording and pacing. A central console that contains the refriger-

ant fluid releases the cryogen under pressure. The cooling liquid travels through the inner delivery lumen to the distal electrode that is maintained under vacuum. At the cryocatheter tip, the liquid cryogen boils. This accelerated liquid-to-gas phase change results in rapid cooling of the distal tip. The gas is then conducted away from the catheter tip via a vacuum return lumen and back to the console where it is collected and restored to its liquid state. Temperature is recorded at the distal tip by an integrated thermocouple device.

Adhesiveness

Several theoretical advantages are noted when cryoablation is compared to radiofrequency (RF) energy, as summarized in Table II. With hypothermia generated at the distal cooling electrode, the catheter adheres to tissue affording greater catheter stability. Metaphorically, this has been likened to the adhesiveness of a wet tongue contacting a frozen pole. Since the catheter is latched on to endocardium, programmed electrical stimulation may be performed during cryoablation without concern for catheter dislodgement. Moreover, “brushing effects” that occur during beat-to-beat rocking heart motions and with respiratory variations are eliminated. This advantage may be particularly profitable if the arrhythmogenic substrate is located at a site where contact is difficult to maintain^{24,25} or ablation of nearby tissue is deemed hazardous. It also permits ablation to be performed during tachycardia without the menace of catheter dislodgement upon abrupt arrhythmia termination.

Table II.
Potential Advantages of Cryoablation over Radiofrequency Ablation

Advantages	Clinical Implications
Catheter adhesiveness	Greater catheter stability Programmed stimulation may be performed during ablation Avoidance of “brushing” effects
Homogeneous sharply demarcated lesion	Less arrhythmogenic More controllable titration of lesion size
Preservation of ultrastructural integrity	Decreased risk of thrombus formation Absence of aneurysmal dilation or rupture
Reversible suppression of conduction tissue	Prediction of successful site Avoidance of unwanted lesions Ablation of high-risk substrates
Lesion limited by warming blood flow Visualization by ultrasound	Safety to nearby epicardial coronary arteries Real-time monitoring Confirmation of endocardial contact Defining optimal freezing parameters
Pain-free ablation	Discomfort minimized under conscious sedation

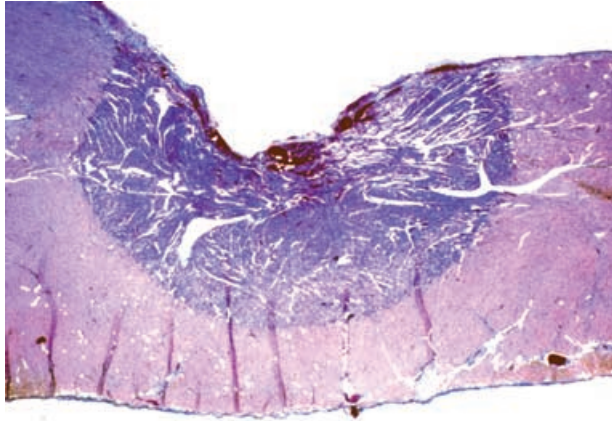


Figure 3. Histological characteristics one week after cryoablation when magnified 16-fold. Note the homogeneous cryolesion with a smooth border, sharp demarcation from intact myocardium, and absence of thrombus. Indentation of the lesion surface arose from mechanical catheter pressure.

Lesion Dimensions

In a preclinical study of 22 mongrel dogs,¹⁴ RF and cryolesion dimensions created by 4-mm-tip catheters were compared. Overall, RF lesions were of greater surface area (42 vs 20 mm², $P = 0.0018$), with nearly significantly larger volumes (95 vs 43 mm³, $P = 0.0585$). Notably, no difference in lesion depth was observed (5 to 6 mm). Histologically, cryolesions were more homogeneous with clearer and smoother demarcations from underlying normal myocardium, as shown in Figure 3. In contrast, RF lesions had rougher more ragged edges. Thus, more focused lesions were noted with cryoablation. Additionally, sharper borders may theoretically be less arrhythmogenic.^{13,65} Border zones with damaged but viable cells are more susceptible to spontaneous depolarization.

Cryoablation dimensions created by 9 versus 7-French catheters were equal in depth but greater in surface area and volume.¹⁴ Colder temperatures were associated with deeper lesions. On average, achieving a peak temperature 10°C colder resulted in a lesion 0.4-mm deeper ($P = 0.0001$). Not unexpectedly, ventricular lesions were deeper than their atrial counterparts and all atrial lesions were transmural. It was therefore demonstrated that larger lesions could be created by reducing the temperature or increasing the surface area of the catheter tip in contact with endocardium.⁶¹ A more recent *in vitro* experiment conducted on porcine ventricular myocardium found that lesion dimensions and tissue temperatures were modulated by convective warming as controlled by su-

perfusate flow, electrode orientation, contact pressure, electrode size, and catheter refrigerant flow rate.⁶⁸ Catheter size modified the effect of electrode temperature on lesion dimensions.

Thrombus Formation

To compare thrombogenesis of RF and cryoenergy ablation, we conducted a randomized preclinical study involving 197 ablation lesions in 22 dogs at right atrial, right ventricular, and left ventricular sites.¹⁴ RF energy was five times more thrombogenic than cryoablation by histological morphometric analyses seven days after ablation. Moreover, thrombus volume was significantly greater with RF compared to cryoablation ($P < 0.0001$). Interestingly, the extent of hyperthermic tissue injury was positively correlated with thrombus bulk. This was unlike cryoenergy, where lesion dimensions were not predictive of thrombus size. It was conjectured that this disparity likely reflected the fact that intact tissue ultrastructure with endothelial cell preservation was maintained with cryoenergy.

Visualization by Ultrasound

In the 1990s, the ability to provide continuous real-time imaging of the freezing process was considered a major technological advancement that sparked renewed interest in visceral cryosurgery.³ Indeed, ultrasonographic monitoring of the freeze/thaw cycle and frozen tissue volume contributed to rapid improvements in hepatic and prostatic surgery. The ability to visualize “ice ball” formation by ultrasonic means was similarly demonstrated in preclinical transcatheter cryoablation studies.¹³ Using a 12.5-MHz rotating transducer mounted on a 6.2-French catheter, intracardiac ultrasound was performed in six dogs who received double freeze/thaw cycles. Endocardial contact was confirmed by echocardiography and serial measurements were made to assess ice ball growth. Intracardiac echocardiography clearly identified ice ball formation as a hypoechogenic density with a bordering hyperechoic rim and posterior acoustic shadowing. No evidence of microcavitation (gas formation) was observed during cryoablation. The size of the ice ball was shown to continuously enlarge during the first three minutes of the freezing cycle and remain stable thereafter. These observations underlie the current recommendation to limit the cryoablation time to four minutes.

Safety to Nearby Epicardial Coronary Arteries

Several concerns have been raised regarding RF ablation near the ostium and within the

coronary sinus. Intracoronary sinus RF ablation damages the vein and may induce fibrosis and stenosis.⁶⁹ Perforation and tamponade are potential complications. However, the most feared adverse event is coronary artery stenosis. The circumflex and/or right coronary artery may course in close proximity to the arrhythmia substrate.^{70–72} Moreover, the AV nodal artery trails near the mouth of the coronary sinus; ablation may conceivably damage this small vessel.⁷³

Preclinical studies suggest a lower incidence of coronary artery stenosis following cryoablation compared to RF ablation. In an experimental study in swine submitted to cryoablation within the mid and distal coronary sinus, no angiographic coronary stenosis was observed and coronary artery medial and intimal layers were preserved.⁷⁴ In a canine model, Aoyama et al.⁷⁵ demonstrated that cryoablation in the coronary sinus within 2 mm of the left circumflex artery produced transmural myocardial lesions similar to RF energy but with a lesser risk of coronary artery stenosis. Histologically, 50% of the animals randomized to RF energy had intimal coronary artery damage compared to none with cryoablation.

Conclusion

The wealth of cellular, preclinical, and clinical experience with surgical cryoablation set the stage for transcatheter cryoablation. Numerous potential advantages were demonstrated in preclinical studies, including enhanced catheter stability, lesser propensity for thrombus formation, temperature titration for reversible effects, ultrasonographic visualization, and delineated focused lesions. Transvenous cryoablation systems were refined as catheter sizes were reduced to the standard 7-French format, steering mechanisms improved, and refrigerants modified to allow more rapid cooling and lower temperatures. Within a relatively brief time span, the initial 9-French steerable catheter with slow cooling and a temperature limit of -50°C was transformed into the modern 7-French version with rapid cooling and achievable temperatures below -75°C . In August 1998, transcatheter cryoablation was first applied to humans.²⁵

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References

- Breasted JH. The Edwin Smith Surgical Papyrus. Chicago: University of Chicago Press, 1980.
- Lustgarten DL, Keane D, Ruskin J. Cryothermal ablation: Mechanism of tissue injury and current experience in the treatment of tachyarrhythmias. *Prog Cardiovasc Dis* 1999; 41:481–498.
- Baust J, Gage AA, Ma H, Zhang CM. Minimally invasive cryosurgery—technological advances. *Cryobiology* 1997; 34:373–384.
- Budman H, Shitzer A, Dayan J. Analysis of the inverse problem of freezing and thawing of a binary solution during cryosurgical processes. *J Biomech Eng* 1995; 117:193–202.
- Whittaker DK. Mechanisms of tissue destruction following cryosurgery. *Ann R Coll Surg Engl* 1984; 66:313–318.
- Gill W, Fraser J, Carter DC. Repeated freeze-thaw cycles in cryosurgery. *Nature* 1968; 219: 410–413.
- Iida S, Misaki T, Iwa T. The histological effects of cryocoagulation on the myocardium and coronary arteries. *Jpn J Surg* 1989; 19:319–335.
- Mikat EM, Hackel DB, Harrison L, Gallagher JJ, Wallace AG. Reaction of the myocardium and coronary arteries to cryosurgery. *Lab Invest* 1977; 37:632–641.
- Tsvetkov T, Tsonev L, Meranzov N, Minkov I. Functional changes in mitochondrial properties as a result of their membrane cryodestruction. II. Influence of freezing and thawing on ATP complex activity of intact liver mitochondria. *Cryobiology* 1985; 22:111–118.
- Mazur P. Cryobiology: The freezing of biological systems. *Science* 1970; 168:939–949.
- Markovitz LJ, Frame LH, Josephson ME, Hargrove WC, 3rd. Cardiac cryolesions: Factors affecting their size and a means of monitoring their formation. *Ann Thorac Surg* 1988; 46:531–535.
- Holman WL, Ikeshita M, Douglas JM, Jr, Smith PK, Cox JL. Cardiac cryosurgery: Effects of myocardial temperature on cryolesion size. *Surgery* 1983; 93:268–272.
- Dubuc M, Roy D, Thibault B, Ducharme A, Tardif JC, Villemarec C, Leung TK, et al. Transvenous catheter ice mapping and cryoablation of the atrioventricular node in dogs. *Pacing Clin Electrophysiol* 1999; 22:1488–1498.
- Khairy P, Chauvet P, Lehmann J, Lambert J, Macle L, Tanguay JF, Sirois MG, et al. Lower incidence of thrombus formation with cryoenergy versus radiofrequency catheter ablation. *Circulation* 2003; 107:2045–2050.
- Cooper IS. Cryogenic surgery: A new method of destruction or extirpation of benign or malignant tissues. *N Engl J Med* 1963; 268:743–749.
- Ott DA, Garson A, Jr, Cooley DA, Smith RT, Moak J. Cryoablative techniques in the treatment of cardiac tachyarrhythmias. *Ann Thorac Surg* 1987; 43:138–143.
- Berth-Jones J, Bourke J, Eglitis H, Harper C, Kirk P, Pavord S, Rajapakse R, et al. Value of a second freeze-thaw cycle in cryotherapy of common warts. *Br J Dermatol* 1994; 131:883–886.
- Pease GR, Wong ST, Roos MS, Rubinsky B. MR image-guided control of cryosurgery. *J Magn Reson Imaging* 1995; 5:753–760.
- Hass GM. A quantitative hypothermal method for the production of local injury of tissue. *Arch Pathol* 1948; 45:563.
- Taylor CB, Davis CB, Jr, Vawter GF, Hass GM. Controlled myocardial injury produced by a hypothermal method. *Circulation* 1951; 3:239–253.
- Harrison L, Gallagher JJ, Kasell J, Anderson RH, Mikat E, Hackel DB, Wallace AG. Cryosurgical ablation of the A-V node-His bundle: A new method for producing A-V block. *Circulation* 1977; 55:463–470.
- Lister JW, Hoffman BF, Kavalier F. Reversible cold block of the specialized cardiac tissues of the unanaesthetized dog. *Science* 1964; 145:723–725.
- Gillette PC, Swindle MM, Thompson RP, Case CL. Transvenous cryoablation of the bundle of His. *Pacing Clin Electrophysiol* 1991; 14:504–510.
- Dubuc M, Talajic M, Roy D, Thibault B, Leung TK, Friedman PL. Feasibility of cardiac cryoablation using a transvenous steerable electrode catheter. *J Interv Card Electrophysiol* 1998; 2:285–292.
- Dubuc M, Khairy P, Rodriguez-Santiago A, Talajic M, Tardif JC, Thibault B, Roy D. Catheter cryoablation of the atrioventricular node in patients with atrial fibrillation: A novel technology for ablation of cardiac arrhythmias. *J Cardiovasc Electrophysiol* 2001; 12:439–444.
- Klein GJ, Sealy WC, Pritchett EL, Harrison L, Hackel DB, Davis D, Kasell J, et al. Cryosurgical ablation of the atrioventricular node-His bundle: Long-term follow-up and properties of the junctional pacemaker. *Circulation* 1980; 61:8–15.

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27. Garratt C, Camm AJ. The role of cryosurgery in the management of cardiac arrhythmias. *Clin Cardiol* 1991; 14:153-159.
28. Bredikis J. Cryosurgical ablation of atrioventricular junction without extracorporeal circulation. *J Thorac Cardiovasc Surg* 1985; 90:61-67.
29. Camm J, Ward DE, Spurrell RA, Rees GM. Cryothermal mapping and cryoablation in the treatment of refractory cardiac arrhythmias. *Circulation* 1980; 62:67-74.
30. Bredikis JJ, Bredikis AJ. Surgery of tachyarrhythmia: Intracardiac closed heart cryoablation. *Pacing Clin Electrophysiol* 1990; 13:1980-1984.
31. Louagie YA, Guiraudon GM, Klein GJ, Yee R. Closed heart cryoablation of the His bundle using an anterior septal approach. *Ann Thorac Surg* 1991; 51:616-619.
32. Gallagher JJ, Sealy WC, Anderson RW, Kasell J, Millar R, Campbell RW, Harrison L, et al. Cryosurgical ablation of accessory atrioventricular connections: A method for correction of the preexcitation syndrome. *Circulation* 1977; 55:471-479.
33. Guiraudon GM, Klein GJ, Gulamhusein S, Jones DL, Yee R, Perkins DG, Jarvis E. Surgical repair of Wolff-Parkinson-White syndrome: A new closed-heart technique. *Ann Thorac Surg* 1984; 37:67-71.
34. Bredikis J, Bredikis A. Cryosurgical ablation of left parietal wall accessory atrioventricular connections through the coronary sinus without the use of extracorporeal circulation. *J Thorac Cardiovasc Surg* 1985; 90:199-205.
35. Rowland E, Robinson K, Edmondson S, Krikler DM, Bentall HH. Cryoablation of the accessory pathway in Wolff-Parkinson-White syndrome: Initial results and long term follow up. *Br Heart J* 1988; 59:453-457.
36. Guiraudon GM, Klein GJ, Sharma AD, Milstein S, McLellan DG. Closed-heart technique for Wolff-Parkinson-White syndrome: Further experience and potential limitations. *Ann Thorac Surg* 1986; 42:651-657.
37. Watanabe S, Koyanagi H, Endo M, Yagi Y, Shiikawa A, Kasanuki H. Cryosurgical ablation of accessory atrioventricular pathways without cardiopulmonary bypass: An epicardial approach for Wolff-Parkinson-White syndrome. *Ann Thorac Surg* 1989; 47:257-264.
38. Lee AW, Crawford FA, Jr, Gillette PC, Roble SM. Cryoablation of septal pathways in patients with supraventricular tachyarrhythmias. *Ann Thorac Surg* 1989; 47:566-568.
39. Gallagher JJ, Anderson RW, Kasell J, Rice JR, Pritchett EL, Gault HJ, Harrison L, et al. Cryoablation of drug-resistant ventricular tachycardia in a patient with a variant of scleroderma. *Circulation* 1978; 57:190-197.
40. Camm J, Ward DE, Cory-Pearce R, Rees GM, Spurrell RA. The successful cryosurgical treatment of paroxysmal ventricular tachycardia. *Chest* 1979; 75:621-624.
41. Krafchek J, Lawrie GM, Roberts R, Magro SA, Wyndham CR. Surgical ablation of ventricular tachycardia: Improved results with a map-directed regional approach. *Circulation* 1986; 73:1239-1247.
42. Page PL, Cardinal R, Shenasa M, Kaltenbrunner W, Cossette R, Nadeau R. Surgical treatment of ventricular tachycardia. Regional cryoablation guided by computerized epicardial and endocardial mapping. *Circulation* 1989; 80:1124-1134.
43. Ott DA, Garson A, Cooley DA, McNamara DG. Definitive operation for refractory cardiac tachyarrhythmias in children. *J Thorac Cardiovasc Surg* 1985; 90:681-689.
44. Guiraudon GM, Thakur RK, Klein GJ, Yee R, Guiraudon CM, Sharma A. Encircling endocardial cryoablation for ventricular tachycardia after myocardial infarction: Experience with 33 patients. *Am Heart J* 1994; 128:982-989.
45. Caceres J, Werner P, Jazayeri M, Akhtar M, Tchou P. Efficacy of cryosurgery alone for refractory monomorphic sustained ventricular tachycardia due to inferior wall infarction. *J Am Coll Cardiol* 1988; 11:1254-1259.
46. Hargrove WC, 3rd, Miller JM, Vassallo JA, Josephson ME. Improved results in the operative management of ventricular tachycardia related to inferior wall infarction. Importance of the annular isthmus. *J Thorac Cardiovasc Surg* 1986; 92:726-732.
47. Miller JM, Marchlinski FE, Harken AH, Hargrove WC, Josephson ME. Subendocardial resection for sustained ventricular tachycardia in the early period after acute myocardial infarction. *Am J Cardiol* 1985; 55:980-984.
48. Cox JL. The status of surgery for cardiac arrhythmias. *Circulation* 1985; 71:413-417.
49. Silka MJ, Kron J, Cutler JE, Wilson RA, Cobanoglu A. Cryoablation of medically refractory nodoventricular tachycardia. *Pacing Clin Electrophysiol* 1990; 13:908-915.
50. Kerr CR, Klein GG, Guiraudon GM, Webb JG. Surgical therapy for sinoatrial reentrant tachycardia. *Pacing Clin Electrophysiol* 1988; 11:776-783.
51. Vermeulen FE, van Hemel NM, Guiraudon GM, Defauw JJ, Elbers HR, de Bakker JM, van Cappelle FJ. Cryosurgery for ventricular bigeminy using a transaortic closed ventricular approach. *Eur Heart J* 1988; 9:979-990.
52. Andress JD, Vander Salm TJ, Huang SK. Bidirectional bundle branch reentry tachycardia associated with Ebstein's anomaly: Cured by extensive cryoablation of the right bundle branch. *Pacing Clin Electrophysiol* 1991; 14:1639-1647.
53. Assad RS, Aiello VD, Jatene MB, Costa R, Hanley FL, Jatene AD. Cryosurgical ablation of fetal atrioventricular node: New model to treat fetal malignant tachyarrhythmias. *Ann Thorac Surg* 1995; 60:S629-S632.
54. Nitta T, Ikeshita M, Asano T, Terada K, Akiyama H, Tanaka S. Perinodal cryomodification for supraventricular tachycardia. *Nippon Kyobu Geka Gakkai Zasshi* 1995; 43:344-349.
55. Szabo TS, Jones DL, Guiraudon GM, Rattes MF, Perkins DG, Sharma AD, Klein GJ. Cryosurgical modification of the atrioventricular node: A closed heart approach in the dog. *J Am Coll Cardiol* 1987; 10:389-398.
56. Klein GJ, Guiraudon GM, Perkins DG, Sharma AD, Jones DL. Controlled cryothermal injury to the AV node: Feasibility for AV nodal modification. *Pacing Clin Electrophysiol* 1985; 8:630-638.
57. Holman WL, Ikeshita M, Lease JG, Smith PK, Lofland GK, Cox JL. Cryosurgical modification of retrograde atrioventricular conduction. Implications for the surgical treatment of atrioventricular nodal reentry tachycardia. *J Thorac Cardiovasc Surg* 1986; 91:826-834.
58. Cox JL, Holman WL, Cain ME. Cryosurgical treatment of atrioventricular node reentrant tachycardia. *Circulation* 1987; 76:1329-1336.
59. Hunt GB, Chard RB, Johnson DC, Ross DL. Comparison of early and late dimensions and arrhythmogenicity of cryolesions in the normothermic canine heart. *J Thorac Cardiovasc Surg* 1989; 97:313-318.
60. Peiffert B, Feldman L, Villemot JP, Verdier J. Cryosurgery in ventricular tachycardia. Value of myocardial hypothermia in the extension of the depth of cryogenic lesions. *Chirurgie* 1992; 118:137-143.
61. Klein GJ, Harrison L, Ideker RF, Smith WM, Kasell J, Wallace AG, Gallagher JJ. Reaction of the myocardium to cryosurgery: Electrophysiology and arrhythmogenic potential. *Circulation* 1986; 73:364-372.
62. Stewart GJ, Preketes A, Horton M, Ross WB, Morris DL. Hepatic cryotherapy: Double-freeze cycles achieve greater hepatocellular injury in man. *Cryobiology* 1995; 32:215-219.
63. Gage AA, Guest K, Montes M, Caruana JA, Whalen DA, Jr. Effect of varying freezing and thawing rates in experimental cryosurgery. *Cryobiology* 1985; 22:175-182.
64. Fujino H, Thompson RP, Germroth PG, Harold ME, Swindle MM, Gillette PC. Histologic study of chronic catheter cryoablation of atrioventricular conduction in swine. *Am Heart J* 1993; 125:1632-1637.
65. Rodriguez LM, Leunissen J, Hoekstra A, Korteling BJ, Smeets JL, Timmermans C, Vos M, et al. Transvenous cold mapping and cryoablation of the AV node in dogs: Observations of chronic lesions and comparison to those obtained using radiofrequency ablation. *J Cardiovasc Electrophysiol* 1998; 9:1055-1061.
66. Hoekstra A, de Langen CD, Niekels PG, Korteling BJ, Bel KJ, Crijns HJ. Prediction of lesion size through monitoring the 0 degree C isothermic period following transcatheter cryoablation. *J Interv Card Electrophysiol* 1998; 2:383-389.
67. Skanes AC, Dubuc M, Klein GJ, Thibault B, Krahn AD, Yee R, Roy D, et al. Cryothermal ablation of the slow pathway for the elimination of atrioventricular nodal reentrant tachycardia. *Circulation* 2000; 102:2856-2860.
68. Wood MA, Parvez B, Ellenbogen AL, Shaffer KM, Goldberg SM, Gaspar MP, Arief I, et al. Determinants of lesion sizes and tissue temperatures during catheter cryoablation. *Pacing Clin Electrophysiol* 2007; 30:644-654.
69. Langberg J, Griffin JC, Herre JM, Chin MC, Lev M, Bharati S, Scheinman MM. Catheter ablation of accessory pathways using radiofrequency energy in the canine coronary sinus. *J Am Coll Cardiol* 1989; 13:491-496.
70. Lemola K, Mueller G, Desjardins B, Sneider M, Case I, Good E, Han J, et al. Topographic analysis of the coronary sinus and major cardiac veins by computed tomography. *Heart Rhythm* 2005; 2:694-699.

71. Becker AE. Left atrial isthmus: Anatomic aspects relevant for linear catheter ablation procedures in humans. *J Cardiovasc Electrophysiol* 2004; 15:809–812.
72. Ho SY, Sanchez-Quintana D, Cabrera JA, Anderson RH. Anatomy of the left atrium: Implications for radiofrequency ablation of atrial fibrillation. *J Cardiovasc Electrophysiol* 1999; 10:1525–1533.
73. Sanchez-Quintana D, Ho SY, Cabrera JA, Farre J, Anderson RH. Topographic anatomy of the inferior pyramidal space: Relevance to radiofrequency catheter ablation. *J Cardiovasc Electrophysiol* 2001; 12:210–217.
74. Skanes AC, Jones DL, Teefy P, Guiraudon C, Yee R, Krahn AD, Klein GJ. Safety and feasibility of cryothermal ablation within the mid and distal coronary sinus. *J Cardiovasc Electrophysiol* 2004; 15:1319–1323.
75. Aoyama H, Nakagawa H, Pitha JV, Khammar GS, Chandrasekaran K, Matsudaira K, Yagi T, et al. Comparison of cryothermia and radiofrequency current in safety and efficacy of catheter ablation within the canine coronary sinus close to the left circumflex coronary artery. *J Cardiovasc Electrophysiol* 2005; 16:1218–1226.