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BASIC RESEARCH

Cryoablation time-dependent dose–response effect at minimal temperatures (−80°C): an experimental study

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Aims To establish a temporal safety window for cryoablation at minimal temperatures and to assess the electrophysiological and histological changes as a function of the application duration.

Methods and results Twenty mini-pigs underwent AV nodal cryoablation at −80°C without prior cryomapping. The duration of the cryoapplication following atrioventricular block (AVB) was randomized to 0, 10, 20, 40, or 60 s. Atrioventricular block was obtained in all animals after a median of 3 (1–8 interquartile range) applications. One week later, AV nodal conduction fully recovered in animals with application duration <10 s, whereas persistent AVB incidence increased as a function of time in animals with longer applications duration. Cryoablation application duration following AVB was the only independent predictor of persistent AVB (OR, 1.116; 95% CI, 1.013–1.229; $P = 0.026$). There was no difference in lesion location or size between animals with vs. those without persistent AVB at 1 week. However, animals randomized to longer application duration demonstrated higher degree of cell destruction and fibrotic content.

Conclusion In this closed-chest pig model, there was a relation between cryoapplication duration following AVB at −80°C and recovery of conduction. A safety window of at least 10 s was observed in all cases.

Keywords Cryothermal ablation • Atrioventricular block • Perinodal ablation • Atrioventricular node • Supraventricular tachycardias

Introduction

Cryothermal ablation is increasingly used in high-risk perinodal ablations, due to the absence of undesired persistent atrioventricular block (AVB) cases reported in the literature.[1] The ability of this technology to create reversible lesions at temperatures of −30°C (cryomapping) is responsible in part for this excellent safety profile.[1–9] However, the electrophysiological effects may not always be predicted by prior cryomapping at −30°C and adverse effects can subsequently be observed during cryoablation (at −70° to −80°C).[2–9] In this case, anecdotal reports suggest that, if the application is immediately interrupted, there is usually complete recovery of conduction.[2–9] We hypothesized that the reversibility of cryothermal application at the AV node is dependent, not only on the minimal temperature at the catheter tip, but also on the duration of the application. Therefore, the

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objectives of this study were to: (i) establish a temporal safety window during AV nodal cryoablation at minimal temperatures (−80°C); (ii) evaluate the residual electrophysiological AV nodal impairment after reversible lesions at −80°C; and (iii) assess the histological changes related to the occurrence of AVB as a function of the duration of the cryoablation application.

Methods

The experimental protocol was approved by the Ethics Committee of Animal Experimentation of our institution. Twenty mini-pigs of either sex weighting 30 ± 4 kg were used in this study.

Electrophysiological study

Three quadripolar catheters were positioned using fluoroscopy to the right atrium, the His bundle, and the right ventricular apex for recording and pacing. Surface electrocardiographic leads and filtered bipolar intracardiac electrograms were recorded at 100 mm/s paper speed. Atrial and ventricular stimulation protocols included pacing trains at a fixed cycle length and the extrastimulus technique.

Atrioventricular conduction properties were assessed at baseline prior to cryoenergy application, 1 h post-ablation and 1 week later. The following parameters were recorded: AH and HV intervals, AV nodal Wenckebach cycle length, AV nodal effective refractory period.

Cryoablation protocol

Percutaneous AV nodal cryoablation was performed using a 9 Fr catheter (Freezor® Max, CryoCath Technologies Inc., Montreal, Canada). The cryocatheter has a distal 8 mm tip cooling electrode and three proximal ring electrodes with 3-5-2 spacing. The principles of the cryoablation system have been described in detail.[1,4,10] The cryoablation catheter was positioned at the triangle of Koch and cryoenergy at minimal temperature of −80°C (without prior cryomapping) was delivered at sites with large low right atrial and small His bundle recordings, aiming to achieve AVB, defined as the occurrence of two consecutive P-waves not conducted to the ventricle. Prior to energy delivery, the duration of the cryoapplication following AVB was randomized to 0, 10, 20, 40, or 60 s (*Figure 1*). The randomization table was designed to obtain equally distributed groups (Group 1: 0 s, Group 2: 10 s, Group 3: 20 s, Group 4: 40 s, Group 5: 60 s). The cryoapplication was maintained until the temperature reached −80°C and stopped if no AVB was obtained 30 s after the minimal temperature was reached. In that case, the catheter was allowed to rewarm and was repositioned to perform another cryoapplication. Afterwards, a permanent pacemaker was implanted and animals were kept alive for 1 week. The outcome variables after cryoablation were: (i) *acute AVB*: complete AVB 1 h after the cryoapplication delivery; (ii) *acute AV conduction recovery*: 1:1 atrial conduction to the ventricle up to 1 h after the cryoapplication delivery; (iii) *delayed AV conduction recovery*: AV conduction recovery that occurred between the end of the procedure up to 1 week later; and (iv) *persistent AVB*: complete AVB 1 week after the cryoablation procedure. The following procedural variables corresponding to the effective cryoapplication (i.e. causing AVB) were recorded.

- *Time to effect*: interval from onset of cryoablation application to AVB occurrence.
- *Temperature at AVB*: catheter tip temperature at the time of AVB occurrence.
- *Nadir temperature*: minimal temperature reached during the application.
- *Time to cryoablation temperature*: interval from onset of cryoablation application to catheter tip temperature ≤ −80°C.
- *Critical cryoablation application duration*: cryoablation application duration interval from onset of AVB until the end of the application.

Histology

Animals were sacrificed at 7 days with a lethal injection of pentobarbital. Their hearts were explanted, rinsed, and fixed in 10% formalin. At least four blocks of tissue of the AV junctions were dehydrated and embedded in paraffin. Specimens were serially sectioned perpendicular to the septal leaflet of the tricuspid valve at a thickness of 7–10μ m with a microtome (Microm HM310) and stained with Masson's trichrome. Cryolesions size was measured using image analysis software (SigmaScanPro 5.0, Jandel Scientific, San Rafael, CA, USA). The geometry of the cryoablation lesions' area was calculated as $A = (1/4)\pi(W/2)D$, and the volume was calculated as $V = (1/2)(4/3)\pi(W/2)^2D$, where W, lesion width and D, lesion depth.[11]

Morphometric analysis at light microscopy was restricted to experiments in which AV conduction block was obtained after a single cryoapplication and to controls. It was performed using digital images (512 × 512 pixels) from each tissue block by using a linear measurement software program (SigmaScan Pro 5.0). Twenty-two measurements of AV nodal and His myocytes diameter (μm) were obtained from each tissue block. For each measurement, the widest cross-sectional myocyte diameter was selected and averaged to allow comparison with controls. To quantify the amount of myocyte/connective tissue, morphometry was performed using a grid of vertical and horizontal lines providing 121 intersections or points. The total number of intersection points was defined as 100%. The proportion of tissue corresponding to connective tissue matrix in each specimen was expressed as connective tissue density, defined as the percentage of intersection points overlying within the tissue extension limited by the grid. Connective tissue density was estimated in a minimum of eight different randomly chosen fields of the AV node and His bundle for each specimen and values were then averaged. Blood vessels and perivascular interstitial tissue were excluded from connective tissue quantification. To quantify the extent of myolysis in the specialized tissue, only myocytes in which the nucleus was present in the plane of the section were considered and at least 12 myocytes per section were analysed. Myocytes were scored by morphometry as: (i) *normal myocytes*, without myolysis, (ii) *mildly myolytic* if myolysis involved between 15 and 35% of the sarcoplasma, and (iii) *severely myolytic* if >40% of the sarcomeres were absent.

In four animals, an electrophysiological study was performed but no cryoapplications were delivered and they were used as control specimens.

Statistical analysis

Results are reported for the entire sample ($n = 20$) unless otherwise specified. Continuous variables are reported as mean ± SD or median and interquartile range (IQR), depending on whether they were normally or non-normally distributed. Categorical variables are reported as number and percentage. Comparisons were made using Student's *t*-test and the Fisher's exact test, as appropriate. In case of non-normally distributed continuous variables, Wilcoxon test was used. Statistically significant variables in the univariate analysis were included in a stepwise logistic regression model. Analyses were performed with SPSS v14.0 and statistical significance was established at $P < 0.05$.

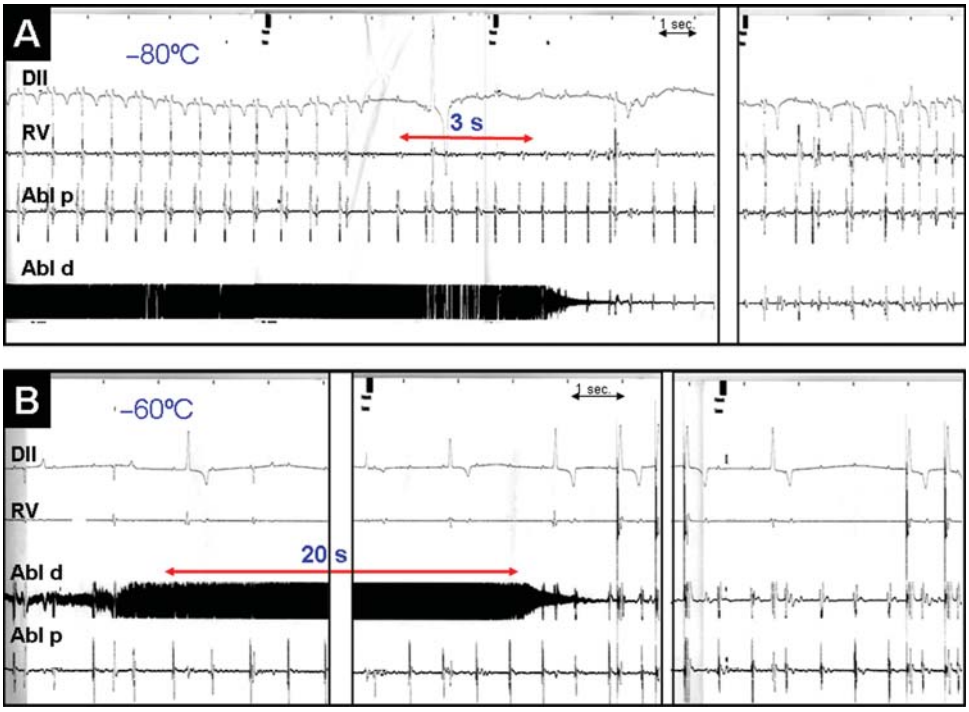

Figure 1 Cryoablation protocol. (*A*) Left: cryoablation application is halted 3 s after AVB occurrence, defined by two consecutive P-waves not conducted to the ventricle, in an animal randomized to 0 s application duration. (*A*) Right: acute AV conduction recovery with resumption of 1:1 atrial conduction to the ventricle 30 s after the cryoapplication was interrupted. (*B*) Left and centre: critical cryoablation application lasting for 20 s, in an animal randomized to 20 s duration application. (*B*) Right: acute AVB 1 h after the cryoapplication delivery. In this case, AVB persisted 1 week after the cryoablation procedure.

Results

A total of 107 cryoablation applications were delivered at the level of the AV node. Atrioventricular block was obtained in all animals after a median of 3 (1–8 IQR) cryoablation applications. A single application was needed to obtain AVB in six (30%) animals, two to four in six (30%), and five or more applications in the remaining. The mean minimal temperature recorded during the applications that caused AVB was -82.3 ± 0.5°C. The median temperature at AVB occurrence was −80°C (range −50 to −85°C). The mean time to effect was 31.8 ± 18.7 s and the mean time to cryoablation temperature ≤ -80°C was 22.2 ± 4.1 s. Mean application duration according to assigned group was: Group 1: 3.75 ± 2.22, Group 2: 14 ± 1.82, Group 3: 22.5 ± 3.32, Group 4: 41.5 ± 12.37, Group 5: 65 ± 6 s.

Atrioventricular conduction acutely recovered in 10 animals after a median of 55 (range 30–1500) seconds following AVB. Two additional animals with acute AVB, had delayed AV conduction recovery 1 week after the ablation procedure. There were no cases of delayed AVB in the group of animals with acute AV conduction recovery following cryoablation. Thus, eight animals had persistent AVB 1 week after the procedure.

Predictors of acute atrioventricular conduction recovery

Atrioventricular nodal conduction fully recovered in all animals randomized to cryoablation application duration of 0 and 10 s following the second-blocked P-wave. No cases of acute AVB occurred with applications duration lasting ≤15 s. In univariate analyses, several variables were associated with the occurrence of acute conduction recovery, whereas lesion size was not (*Table 1*). In multivariate analysis, critical cryoablation application duration from AVB onset remained as the only significant predictor of acute AV conduction recovery (OR, 0.887; 95% CI, 0.799–0.984; $P = 0.024$).

Predictors of persistent atrioventricular block occurrence

One week after the cryoablation procedure, there were no cases of persistent AVB in animals randomized to application duration of 0 and 10 s following the second-blocked P-wave, whereas the probability of presenting persistent AVB increased as a function of time in animals with longer application duration. Persistent AVB occurred in one (25%), three (75%), and four (100%) animals assigned to critical cryoapplication duration of 20, 40, and 60 s, respectively. The first case of persistent AVB occurred after a 16 s application duration following the second-blocked P-wave. Several variables were related to the occurrence of persistent AVB in the univariate analyses (*Table 1*). In a multiple logistic regression model, critical application duration from AVB onset was the only independent predictor of persistent AVB (OR, 1.116; 95% CI, 1.013–1.229; $P = 0.026$).

Table 1 Univariate predictors of acute atrioventricular nodal conduction recovery and persistent atrioventricular block at 1 week

	Acute AV nodal conduction recovery		*P*-value	Persistent AVB at 1 week		*P*-value
	Yes ($n = 10$)	No ($n = 10$)		No ($n = 12$)	Yes ($n = 8$)	
Number of applications	6 (11)	3 (3)	0.131	3.5 (10)	3 (6)	0.635
Time to effect	40 (32)	19 (18)	0.016	34.5 (31)	19 (24.3)	0.031
Time to cryoablation temperature	21.55 ± 5.14	23 ± 4.27	0.507	21.83 ± 5.32	22.75 ± 3.88	0.662
Critical cryoablation application duration	13 (18)	49 (42.5)	0.003	14 (17.75)	48 (33.25)	0.010
Temperature at AVB	−80 (0)	−80 (13)	0.045	−80 (0)	−78.5 (14)	0.037
Nadir temperature	−83 (5)	−82 (3.5)	0.614	−82.5 (4.5)	−82 (4.25)	0.694
Lesion depth	2.95 (0.7)	3 (1.85)	0.682	2.9 (0.5)	3.3 (2.35)	0.281
Lesion area	16.05 ± 7.67	14.7 ± 8.56	0.724	14.94 ± 6.85	16.06 ± 9.63	0.768
Lesion volume	39.45 ± 22.67	40.91 ± 24.63	0.894	40.38 ± 23.15	40 ± 24.56	0.973

Mean ± SD; median (interquartile range).

Table 2 Atrioventricular nodal conduction characteristics before and following cryoablation in animals with atrioventricular conduction recovery

	Baseline	Acute ($n = 10$)	*P*-value[a]	1 Week ($n = 12$)	*P*-value[b]
AH interval	81.1 ± 8.2	91.1 ± 12.7	0.04	93.6 ± 11.2	0.04
HV interval	32.2 ± 5.6	32.8 ± 6.8	ns	32.3 ± 6.5	ns
Wenckebach cycle	271.1 ± 38.9	268.9 ± 50.8	ns	250.9 ± 43.5	ns
AVN refractory period 600	277.8 ± 32.3	297.8 ± 33.1	ns	283.6 ± 38.3	ns
AVN refractory period 400	245 ± 30.1	223.7 ± 93.5	ns	251 ± 28.1	ns

[a]Baseline vs. acute.
[b]Baseline vs. 1 week.

Atrioventricular nodal conduction characteristics following transient atrioventricular block

A significant prolongation of the AH interval was the only electrophysiological parameter that was affected by the cryoablation application in animals with AV nodal conduction recovery (*Table 2*). Other parameters of AV nodal function fully normalized at 60 min and at 7 days after cryoablation.

Histology

On qualitative analysis, all cryoablated specimens were consistently characterized by extensive myocellular damage involving the AV junction walls. Cryolesions were well-circumscribed discrete lesions with sharp borders, dense areas of fibrotic tissue, and contraction band necrosis (*Figure 2*). Myocardial tissue displayed shrunk cells with degenerated cytoplasms, decreased staining, coagulation necrosis, lymphocytes, and macrophages infiltration (*Figure 3*). Overall ($n = 20$), there was no difference in lesion size between animals with vs. without AVB acutely or 1 week after the cryoablation procedure (*Table 1*).

In six animals, AV conduction block was obtained after a single cryoapplication. Of those, AV nodal conduction acutely recovered in animals randomized to application duration of 0 and 10 s ($n = 2$), whereas AVB persisted >1 h after the procedure in the remaining animals randomized to 20–60 s application duration (*Table 3*). Histologically, all six animals demonstrated a lesion that affected the proximal AV conduction system, both the penetrating bundle and a portion of the AV node. There was no difference in lesion location at the AV node level or in lesion size between animals with AV nodal conduction recovery vs. persistent AVB at 1 week (*Figure 2*). Morphometric analysis of the cryolesions showed loss of cellular viability throughout the wall thickness. However, in animals that subsequently had acute AV conduction recovery (#6, #7), isolated viable cells surrounded by necrotic tissue were observed within the lesions of the specialized AV node and penetrating His bundle (*Figure 3*). Moreover, the degree of myolytic changes differed between animals with vs. without persistent AVB at 1 week (*Table 3* and *Figure 3*). Animals with AV conduction recovery showed only mild myolytic changes, whereas severe myolytic changes (>40% myolytic fibres), both at the AV node level and the penetrating His bundle, were observed in animals with persistent AVB (#3, #19, #22). Similarly, animals with persistent AVB at 1 week demonstrated higher collagen content when compared with animals with AV

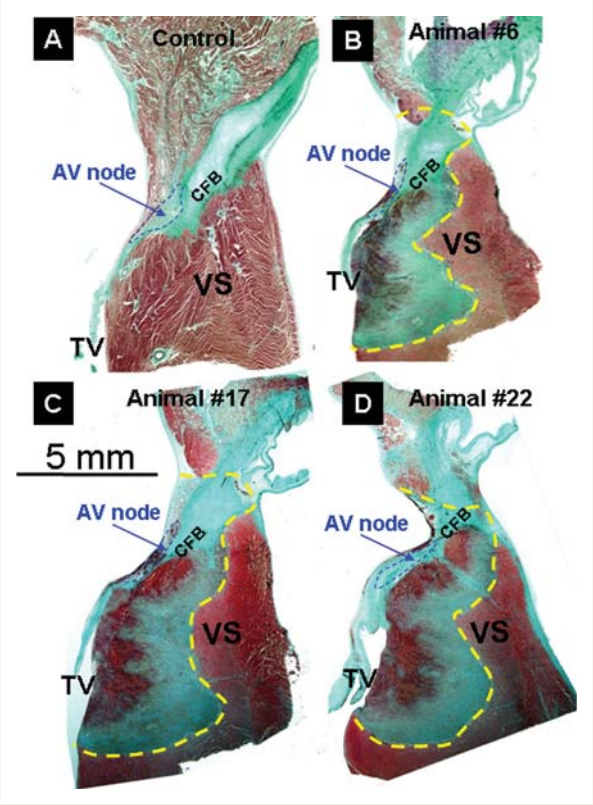

Figure 2 Cryolesions histological characteristics at the AV node level. Control experiment (*A*) and cryolesion histological characteristics at the AV node level in three specimens (*B*–*D*) 1 week after a single cryoenergy application that produced AVB (dotted blue line = AV node), when stained with Masson's trichrome. Note shape of the lesion resembling a prolate hemisphere (dotted yellow line) and the homogeneous nature of cryolesion, with a smooth and sharp demarcation from intact myocardium, with replacement of muscle by granulation tissue at perimeter of the lesion and coagulative necrosis of underlying myocardium. TV, tricuspid valve; CFB, central fibrous body; VS, ventricular septum.

conduction recovery and controls. Interestingly, case #17 had delayed AV conduction recovery at 1 week and showed intermediate myolytic changes and fibrotic content level when compared with animals with acute recovery and persistent AVB. Finally, a progressive increase in cell size was observed from controls to persistent AVB animals.

Discussion

The main findings of the present study are that in this closed-chest pig model: (i) there is a safety window of at least 10 s during cryoablation at minimal temperatures (−80°C), where complete recovery of the AV node electrophysiological properties is always observed; (ii) the main determinant of the electrophysiological effects of cryoablation of the AV node at minimal temperatures is application duration, but not lesion size; (iii) changes at the microscopic level, such as cell destruction and fibrosis, explain the irreversible electrophysiological effects demonstrated by applications duration lasting >15 s.

Undesired atrioventricular block during cryoablation at −80°C

As opposed to radiofrequency ablation, there are no cases reported in the literature of undesired persistent AVB during cryoablation.[1–9] For this reason, cryoablation has become a very attractive alternative to radiofrequency during perinodal ablations, particularly in young individuals in whom AVB would be a disastrous outcome. In this setting, cryomapping at −30°C creates reversible lesions that enable testing the functional effects of ablation before the formation of a permanent lesion.[1,2,10] However, the electrophysiological effects may not always be predicted by prior cryomapping at −30°C and adverse effects can be observed during cryoablation (at −70 to −80°C).[2–9] We and others have reported the occurrence of transient high-grade AVB during cryoablation at sites where previous cryomapping was judged to be 'safe' as verified using a navigation system.[2,7] Although gradual normalization of the electrophysiological properties has been observed when the application is immediately interrupted, no information exists to date regarding the safety profile of cryoablation in this situation.

Application duration as a determinant of the electrophysiological effect

Permanence of tissue destruction with cryothermal energy is related to time-dependent variables and tissue temperature.[1,9,12–15] Holman *et al.*[16] first reported the occurrence of undesired AVB during selective cryosurgical ablation (−60°C) of the perinodal tissue that completely resolved over time if the cryothermic exposure was immediately terminated. They also found that despite prolongation of AH and VA intervals, Wenckebach point, and AV nodal effective refractory period in the immediate post-operative period, all these variables normalized by 14 days after surgery except for the AH interval. In our study, only a significant prolongation in the AH interval following transient AVB at −80°C was observed acutely and 1 week later. Also in agreement with them, no instances of persistent AVB were observed during follow-up in our study after applications lasting <16 s.

Several clinical studies in humans have reported the occurrence of undesired transient AVB during cryoablation applications at temperatures ≤−70°C.[2–9] In all cases AVB resolved completely, usually within a few seconds or minutes, with PR interval and Wenckebach point returning to baseline. In those studies, no patient had persistent AVB or required pacemaker implantation. The present study confirms the benign outcome of undesired complete AVB that occurs during cryoablation at −80°C. Moreover, the observation that there were two cases of delayed AV conduction recovery and no cases of AV nodal conduction impairment 1 week following cryoablation suggests that long-term deterioration is unlikely. However, although either acute or delayed AV nodal conduction recovery can be observed with applications lasting >15 s, the probability of persistent AVB occurrence significantly increased over that application duration, indicating

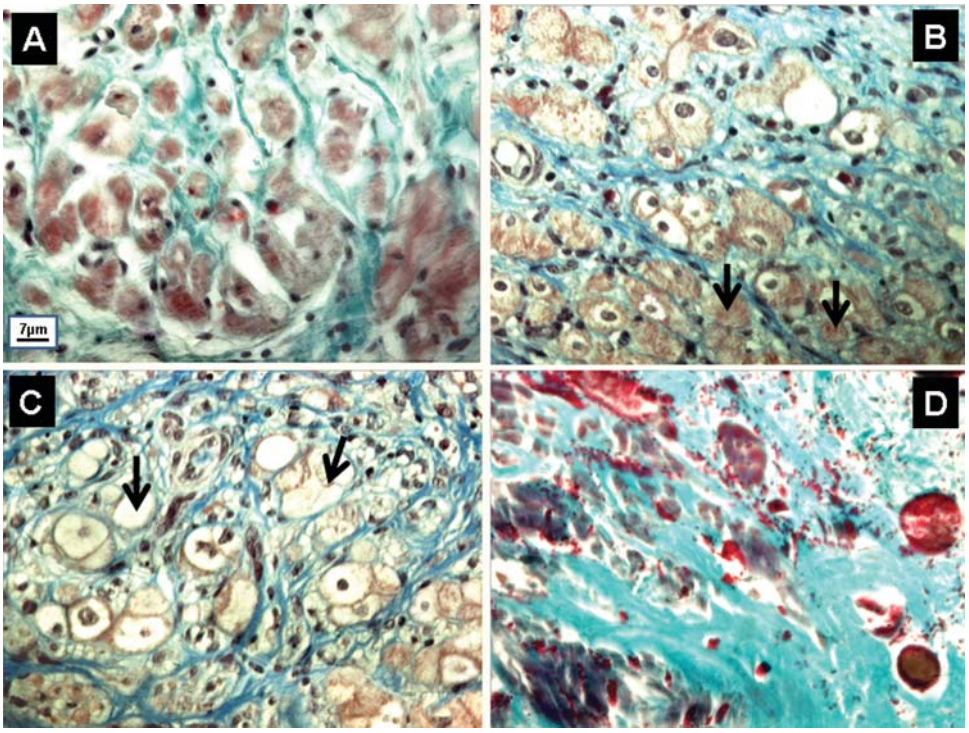

Figure 3 AV nodal myocytes characteristics after a single cryoenergy application in relation to AVB occurrence at 1 week (corresponds to *Figure 2* magnification at the AV node level). AV nodal myocytes in control specimen (*A*) and after a single cryoenergy application in relation to AVB occurrence at 1 week (*B*–*D*). (*A*) Control specimen, showing normal AV nodal cells. (*B*) Isolated viable cells (arrows) surrounded by necrotic cells in animal #6 (acute AV recovery). (*C*) Moderate degenerative changes characterized by loss of contractile material (arrows) and mild collagen infiltration in animal #17 (delayed AV conduction recovery at 1 week). (*D*) Severe myolytic changes and dense collagenous scar with coagulative necrosis in animal #22 (persistent AVB).

that, in fact, permanent AV nodal block occurs with long enough cryoenergy applications.

Other authors have failed to obtain persistent AVB in a significant proportion of patients with clinical indication of AV node ablation, despite delivering long duration cryoenergy applications preceded by multiple cryomapping attempts.[4,14] Several circumstances may explain their results, such as smaller catheter tip size (4–6 mm), catheter tip–heart size mismatch, tissue oedema as a consequence of multiple consecutive cryomapping and cryoablation attempts, and higher temperatures during cryoablation.[4,14] Our results confirm the ability of long enough cryoablation applications (>60 s) to consistently produce persistent AVB when large catheters (8 mm) are used and very low temperatures (<−80°C) are reached.

Characteristics of the cryolesion

Several hypotheses have been proposed to explain the reversibility of the electrophysiological effects during cryoablation such as iceball enlargement and temperature gradient effect on tissues in the proximity of the catheter tip, but none has systematically analysed the histological correlates of reversibility of the electrophysiological effects.[8,9] In the present *in vivo* study using cryoablation at minimal temperatures (−80°C), we found no differences in lesion location or in lesion size between animals with vs. those without persistent AVB, application duration being the only independent predictor of AV nodal conduction recovery. Separate analysis of experiments in which AVB was obtained with a single application confirmed these results.

At minimal temperatures, cryothermal energy created a well-defined lesion with sharp borders displaying the characteristic features previously described (*Figure 2*).[11–13,16,17] We performed morphometric analysis of the lesions to get further insights into the factors determining normalization of the electrophysiological conduction properties. The degree of myolytic changes and collagen content of the lesion increased as a function of the critical cryoapplication duration, thereby determining the electrophysiological effect. Interestingly, strands of isolated viable cells surrounded by necrotic tissue were observed within the lesions of the specialized conduction system in animals that subsequently showed AV conduction recovery (*Figure 3*). Similar findings were observed by Fujino *et al.*[17] in animals demonstrating late AV conduction recovery at 6 weeks following percutaneous AVB cryoablation attempts. Thus, applications lasting <15 s from the electrophysiological effect onset create a well-defined lesion, but enable some viable cells to survive. However, the long-term outcome of these lesions remains unknown and studies are needed to establish the risk of delayed (>1 week) AVB development under these conditions.

Table 3 Cryolesions morphometric analysis at light microscopy in experiments (#) with atrioventricular block after a single cryoapplication

Experiment #	Randomized group assignment	Critical application duration (s)	AVN conduction after ablation	Myocyte diameter (μm)	CTD/field (%)	Myolysis AV node/field (%)	Myolysis His bundle/field(%)
Controls $n = 4$	NA	NA	NA	7.5 ± 1.2 (5–9)	20 ± 4 (24–35)	8 ± 2 (5–12)	7 ± 3 (3–12)
#7	Group 1	1	Acute AV conduction recovery	12 ± 2 (7–18)	35 ± 4 (28–45)	21 ± 4 (16–29)	25 ± 4 (18–35)
#6	Group 2	15	Acute AV conduction recovery	13 ± 2 (8–18)	30 ± 4 (23–46)	25 ± 4 (19–33)	22 ± 3 (18–40)
#17	Group 3	23	Delayed AV conduction recovery	15 ± 2 (9–20)	40 ± 4 (33–50)	35 ± 7 (25–48)	32 ± 4 (26–43)
#3	Group 3	20	Persistent AVB	15 ± 2 (10–22)	58 ± 4 (48–75)	65 ± 5 (55–85)	68 ± 5 (55–95)
#19	Group 5	60	Persistent AVB	17 ± 2 (9–24)	62 ± 4 (50–80)	60 ± 5 (55–85)	66 ± 5 (55–98)
#22	Group 5	60	Persistent AVB	16 ± 2 (9–20)	65 ± 5 (50–83)	77 ± 5 (65–98)	85 ± 5 (72–98)

Mean ± SD (range). AVB, atrioventricular block; CTD, connective tissue density; NA, not applicable.

Limitations

Several limitations in our work should be discussed. First, lesion size can depend on several conditions such as electrode orientation, contact pressure, and blood velocity that were not controlled for in the present study.[12,13] For this reason, application duration was randomly assigned to partially overcome this limitation. Second, more than one cryoablation application was needed to obtain AVB in two-thirds of the experiments. To avoid bias caused by the possibility of lesion confluence and the effect of consecutive freeze–thaw cycles, morphometric analysis was limited to experiments in which AVB was obtained with a single application. Nevertheless, ineffective applications are most likely due to inadequate position not affecting the AV node[17] and, in most cases, consecutive cryolesions could be individualized in our study. Finally, longer term observations in humans are needed to confirm the favourable outcome of reversible lesions created at minimal temperatures in this model.

Clinical implications

More aggressive strategies are used with the aim of increasing success rates and reducing recurrences during cryoablation. In patients with atrioventricular nodal reentrant tachycardia, larger tip cryoablation catheters are associated with fewer recurrences in long-term follow-up.[8] Similarly, in patients undergoing AV nodal therapeutic ablation, long lasting applications using large catheters (i.e. 8 mm) and catheter tip temperatures $\leq -80^{\circ}C$ should be needed to consistently obtain persistent AVB. In patients with accessory pathways, it has been suggested that substituting cryomapping by direct cryoablation controlling 'time to success' can achieve a high success rate.[9] Our study suggests that, even using low temperatures ($-80^{\circ}C$) and large tip electrodes, no persistent AVB could be expected during perinodal cryoablation if the application duration is halted within 10 s of AVB onset. These results might be useful in further defining the role of this energy for ablation of arrhythmic substrates at high risk of AVB.

Conflict of interest: F.A. served as consultant to the Medtronic European Advisory Board; F.A. and J.A. received research grant support from St. Jude Medical, Spain; the other authors report no conflicts of interest.

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References

1. Skanes AC, Klein G, Krahn A, Yee R. Cryoablation: potentials and pitfalls. *J Cardiovasc Electrophysiol* 2004;**15**:S28–34.
2. Atienza F, Arenal A, Torrecilla EG, García-Alberola A, Jiménez J, Ortiz M *et al.* Acute and long-term outcome of transvenous cryoablation of midseptal and parahissian accessory pathways in patients at high risk of atrioventricular block during radiofrequency ablation. *Am J Cardiol* 2004;**93**:1302–5.
3. Gaita F, Haissaguerre M, Giustetto C, Grossi S, Caruzzo E, Bianchi F *et al.* Safety and efficacy of cryoablation of accessory pathways adjacent to the normal conduction system. *J Cardiovasc Electrophysiol* 2003;**14**:825–9.

4. Friedman PL, Dubuc M, Green MS, Jackman WM, Keane DTJ, Marinchak RA *et al.* Catheter cryoablation of supraventricular tachycardia: results of the multicenter prospective 'frosty' trial. *Heart Rhythm* 2004;**1**:129–38.
5. Fischbach PS, Saarel EV, Dick M II. Transient atrioventricular conduction block with cryoablation following normal cryomapping. *Heart Rhythm* 2004;**1**:554–7.
6. Kimman GP, Theuns DAMJ, Szili-Tovok T, Scholten MF, Res JC, Jordaens LJ. CRAVT: a prospective, randomized study comparing transvenous cryothermal and radiofrequency ablation in atrioventricular nodal reentrant tachycardia. *Eur Heart J* 2004;**25**:2232–7.
7. Kriebel T, Broistedt C, Kroll M, Sigler M, Paul T. Efficacy and safety of cryoenergy in the ablation of atrioventricular reentrant tachycardia substrates in children and adolescents. *J Cardiovasc Electrophysiol* 2005;**16**:960–6.
8. Rivard L, Dubuc M, Guerra PG, Novak P, Roy D, Macle L *et al.* Cryoablation outcomes for AV nodal reentrant tachycardia comparing 4-mm versus 6-mm electrode-tip catheters. *Heart Rhythm* 2008;**5**:230–4.
9. Kaltman JR, Tanel RE, Wegrzynowicz B, Kozodoy E, Wieand T, Ennis J *et al.* Time and temperature profile of catheter cryoablation of right septal and freewall accessory pathways in children. *J Cardiovasc Electrophysiol* 2008;**19**:343–7.
10. Dubuc M, Khairy P, Rodriguez-Santiago A, Talajic M, Tardif MJ, Thibault B *et al.* Catheter cryoablation of the atrioventricular node in patients with atrial fibrillation: a novel technology for ablation of cardiac arrhythmias. *J Cardiovasc Electrophysiol* 2001;**12**:439–44.
11. Wadhwa M, Rahme MM, Dobak J, Li H, Wolf P, Chen P *et al.* Transcatheter cryoablation of ventricular myocardium in dogs. *J Interv Card Electrophysiol* 2000;**4**: 537–45.
12. Tse HF, Ripley KL, Lee KL, Siu CW, Van Vleet JF, Pelkey WL *et al.* Effects of temporal application parameters on lesion dimensions during transvenous catheter cryoablation. *J Cardiovasc Electrophysiol* 2005;**16**:201–4.
13. Parvez B, Pathak V, Schubert CM, Wood MA. Comparison of lesion sizes produced by cryoablation and open irrigation radiofrequency ablation catheters. *J Cardiovasc Electrophysiol* 2008;**19**:528–34.
14. Perez-Castellano N, Villacastín J, Moreno J, Isa R, Ruiz E, Doblado M *et al.* High resistance of atrioventricular node to cryoablation: a great safety margin targeting perinodal arrhythmic substrates. *Heart Rhythm* 2006;**3**:1189–95.
15. Chun KR, Fürnkranz A, Metzner A, Schmidt B, Tilz R, Zerm T *et al.* Cryoballoon pulmonary vein isolation with real-time recordings from the pulmonary veins. *J Cardiovasc Electrophysiol* 2009; Epub ahead of print 13 July 2009, doi: 10.1111/j.1540-8167.2009.01549x.
16. Holman WL, Hackel DB, Lease JG, Ikeshita M, Cox JL. Cryosurgical ablation of atrioventricular nodal reentry: histologic localization of the proximal common pathway. *Circulation* 1988;**77**:1356–62.
17. 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**:1632–7.