Catheter ablation has emerged as a powerful treatment modality for several different cardiac arrhythmias, including atrial fibrillation, ventricular tachycardia, and atrioventricular node ablation. However, ablation often lacks efficacy; this may be because of reconnection of the pulmonary veins or involvement of further atrial substrate, driving the recurrence of the arrhythmia in atrial fibrillation patients. Efficacy of ventricular tachycardia ablation is often limited due to difficulty of creation of full-thickness lesions in the ventricles, because of limited tissue penetration with radiofrequency energy. Catheter ablation requires extensive training, can be tedious, and also carries risk of several complications, such as thrombembolic events, pulmonary vein stenosis, and often lethal atrial-esophageal fistula formations.1

Invasive delivery of β-irradiation to distinct arrhythmogenic cardiac locations has demonstrated feasibility and created enthusiasm for generation of myocardial lesions using ionizing radiation.2–4 Noninvasive delivery of x-rays using stereotactic

**Original Article**

**Atrioventricular Node Ablation in Langendorff-Perfused Porcine Hearts Using Carbon Ion Particle Therapy**

**Methods and an In Vivo Feasibility Investigation for Catheter-Free Ablation of Cardiac Arrhythmias**

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**Background**—Particle therapy, with heavy ions such as carbon-12 (12C), delivered to arrhythmogenic locations of the heart could be a promising new means for catheter-free ablation. As a first investigation, we tested the feasibility of in vivo atrioventricular node ablation, in Langendorff-perfused porcine hearts, using a scanned 12C beam.

**Methods and Results**—Intact hearts were explanted from 4 (30–40 kg) pigs and were perfused in a Langendorff organ bath. Computed tomographic scans (1 mm voxel and slice spacing) were acquired and 12C ion beam treatment planning (optimal accelerator energies, beam positions, and particle numbers) for atrioventricular node ablation was conducted. Orthogonal x-rays with matching of 4 implanted clips were used for positioning. Ten Gray treatment plans were repeatedly administered, using pencil beam scanning. After delivery, positron emission tomography-computed tomographic scans for detection of β+ (11C) activity were obtained. A 12C beam with a full width at half maximum of 10 mm was delivered to the atrioventricular node. Delivery of 130 Gy caused disturbance of atrioventricular conduction with transition into complete heart block after 160 Gy. Positron emission computed tomography demonstrated dose delivery into the intended area. Application did not induce arrhythmias. Macroscopic inspection did not reveal damage to myocardium. Immunostaining revealed strong γH2AX signals in the target region, whereas no γH2AX signals were detected in the unirradiated control heart.

**Conclusions**—This is the first report of the application of a 12C beam for ablation of cardiac tissue to treat arrhythmias. Catheter-free ablation using 12C beams is feasible and merits exploration in intact animal studies as an energy source for arrhythmia elimination. (Circ Arrhythm Electrophysiol. 2015;8:429-438. DOI: 10.1161/CIRCEP.114.002436.)

**Key Words:** atrial fibrillation ◼ atrioventricular node ◼ carbon particle therapy ◼ catheter-free ablation ◼ tachycardia, ventricular
WHAT IS KNOWN

- Carbon ion (\(^{12}\text{C}\)) particle therapy is an extremely focused form of ionizing radiation that can be delivered to deeply situated targets with little beam entry and almost no beam exit dose.
- \(^{12}\text{C}\) has proven to be highly effective for treatment of several cancer types.

WHAT THE STUDY ADDS:

- Catheter-free ablation with \(^{12}\text{C}\) dose deposition into the atrioventricular node can be successfully achieved, despite cardiac motion interplay on the ion beam.
- Catheter-free arrhythmia ablation using \(^{12}\text{C}\) is feasible and merits exploration in intact animal studies.

Carbon ion (\(^{12}\text{C}\)) particle therapy has lately created incentive for a catheter-free ablation approach.\(^6\) Sophisticated beam deliveries have gained some accuracy for lesion creation, minimizing high doses to surrounding tissues. However, these techniques expose high-tissue volumes to low doses of irradiation.\(^6\)

Accelerated hadrons, such as protons as well as heavy charged particles as carbon-12 ions (\(^{12}\text{C}\)), are marked by unique physical properties with maximal dose deposition in form of the Bragg peak.\(^7\) This allows superior precision of the energy delivery with a low entrance and almost no exit dose.\(^7,8\) \(^{12}\text{C}\), in addition, has a smaller lateral spread than protons and deposits 24x more energy into a cell than x-rays and protons.\(^9\)

We hypothesize that accelerated particles can be used as a new catheter-free approach for ablation of cardiac tissue and hence arrhythmia elimination. This investigation sought to provide feasibility data as a foundation for subsequent intact animal studies using a raster-scanned \(^{12}\text{C}\) beam for focused ablation of myocardium.

Methods

General Methods

In this feasibility study, we decided for a Langendorff-setup to investigate acute beam effects in a high-dose escalation model. In addition, we used this setup to uniquely focus on cardiac motion for evaluation of local ion beam interplay without consideration of respiratory motion. Finally, this setup was used to assess the first steps in the lesion cascade using \(^{12}\text{C}\).

Animals

Four domestic pigs with a weight of 30 to 40 kg were studied using Langendorff preparations. All animal procedures were approved by the institutional ethics committee of the University of Heidelberg and the regional board of the state of Baden-Württemberg, Karlsruhe, Germany.

Heart Extractions

Heart extractions were performed as described elsewhere.\(^10\) Briefly, after a deep level of anesthesia was reached, a right lateral thoracotomy was performed. Heparin and 1 to 1.5 L of cardioplegia (Bretschneider cardioplegia) were administered. After asystole was obtained, the heart was removed from the chest and placed into a bath of 5°C to 7°C cold cardioplegia. Selective cannulation of the left main and right coronary artery was performed (cannula Ø 4 mm, length 5 mm/10 mm, left main and right coronary artery, respectively) and fiducial clips were placed in the coronary sinus ostium, noncoronary cusp of the aorta, right atrial appendage, and left atrial appendage. Subsequently, hearts were transferred in cold cardioplegic solution from the extraction site to the Heidelberg Ion Therapy Center.

Langendorff Perfusion

The system consisted of a roller pump (Ismatec, Wertheim, Germany) with divided circuits for right and left coronary perfusion, allowing precise flow adjustments in each circuit. Reperfusion was initiated with a warmed (37°C) normokalemic modified Krebs–Henseleit buffer consisting of Na-glucose 2.0 g/L, MgCl, 0.141 g/L, KHPO\(_4\), 0.16 g/L, KCI 0.35 g/L, NaCl 6.99 g/L, NaHCO\(_3\), 0.73 g/L, CaCl\(_2\), modified with 0.125 g/L bovine albumin (Sigma Aldrich, St. Louis, MO), oxygenated with 95% O\(_2\)/5%CO\(_2\) (≈2 L/min) through a membranous oxygenator. Subsequently, hearts were placed in an externally heated water-jacketed organ bath of polymethyl-methacrylate to maintain temperature. A volume of ca. 3-L Krebs–Henseleit buffer was circulated. Solution was filtered and exchanged at an average rate of 1.5 L/hr. Perfusion of each coronary side was flow controlled, with constant monitoring of the perfusion pressure (target pressure, 80–120 mm Hg [SA monitor 1.2; SA Instruments, Inc, NY]). Blood gas analyses were performed every 30 minutes with all variable perfusion parameters accordingly adjusted.

Specific Methods

Treatment Planning Computed Tomographic Acquisition

For acquisition of a treatment planning computed tomographic (CT) scan, a nongated, non–contrast-enhanced CT scan with 1-mm voxel and 1-mm reconstructed slice spacing, respectively, was acquired on a multidetector (64 row each) 128 dual processor Siemens Somatom Definition Flash scanner with positron emission tomography (PET) capability through a Biograph TruePoint (Siemens Healthcare, Forchheim, Germany).

CT Contouring: Target Identification

For targeting of the atrioventricular node, a 5-mm sphere forming the target volume was contoured between the fiducial clip of the CS ostium and the clip of the aortic noncoronary cusp of the aorta. Here, all 3 multiplanar reconstructions of the acquired CT scan were used to determine the target location. Subsequently, the clinical target volume was extended via addition of 10-mm isotropic (equal in the x, y, and z dimensions) margin. This technique was used to accommodate for target motion as well as uncertainties in positioning of the ex situ heart.

Carbon Ion Beam Treatment Planning

The treatment planning software Syngo PRT Planning (VC11B) application (Siemens AG, Erlangen, Germany) was used. Single horizontal ion beam line treatment plans were created. Treatment plans enable precise beam deposition into the target and thus include calculations of the number of particles for each CT voxel. Therefore, matching of the heart setup to ensure exact concordance between pretreatment CT scan and the treatment position is crucial. In this study, an x-ray–based digital positioning system with matching of the pretreatment CT scan and the treatment position is crucial. In this study, an x-ray–based digital positioning system with matching of the pretreatment CT scan and the treatment position is crucial. In this study, an x-ray–based digital positioning system with matching of 2 acquired orthogonal image planes to 2 orthogonal CT deriving precision flash radiographs was used (Siemens AG, Erlangen, Germany). Delivery of 70, 90, and 160 Gy was picked to elicit acute electrophysiological reactions on beam delivery if present.

Carbon Ion Beam Delivery

\(^{12}\text{C}\) ion radiotherapy was performed at the Heidelberg Ion-Beam Therapy Center, Heidelberg, Germany, with 1 horizontal beam line using the raster scanning technique developed by Haberer et al.\(^11\) Here, \(^{12}\text{C}\) ions coming from a CO\(_2\) source are preaccelerated by a
linear accelerator. Subsequently, a synchrotron accelerates $^{12}$C to energies of 88 to 430 MeV/u. The energy used in the synchrotron for ion acceleration varies the position of the point of maximum energy release, Bragg peak, and thus leads to dose deposition at different beam penetration depths. For delivery, the target volume (in the CT scan) is divided into multiple slices (calculated during treatment planning), where each slice corresponds to 1 energy level of accelerated ions. At each slice, the beam is also deviated in the x and y direction, covering these 2 dimensions of the target as well. This so-called pencil beam scanning enables true 3-dimensional dose painting of the particle beam into the target volume.

Electrogram and Cardiac Rhythm Monitoring
Cardiac electrograms were continuously acquired via an amplifier for isolated hearts (Hugo Sachs Elektronik, March, Germany). The digital signal was conducted out of the treatment room and simultaneously stored, allowing real-time and off-line analysis. At baseline, and whenever appropriate, Wenckebach periods were assessed using a cardiac stimulator using 2 unipolar pacing electrodes sutured to the right atrial appendage (HA Stimulator P, Hugo Sachs Elektronik, March, Germany).

PET-CT Acquisition
During a time window of 20 to 30 minutes after completion of the irradiation protocol, PET-CT scanning for detection of $\beta^+$ emitters, mainly $^{12}$C (by-product of heavy ion therapy) was conducted. Subsequently, this allowed correlation of measured positron tissue activity to the predicted activity from the treatment plan. For acquisition a Siemens Biograph TruePoint PET-CT scanner (Siemens Healthcare, Forchheim, Germany) was used and concordance analysis between the treatment plan and the measured topical PET activity was performed.

Pathohistological Examination
A detailed pathohistological examination was performed on all hearts. Tissue was obtained from all areas exposed to $^{12}$C in the target area, beam entry channel, and myocardium out of the beam field. Control samples of the heart which was not irradiated were also isolated. For histological analysis samples were fixed in 4% formaldehyde and processed as described. Samples for protein extraction were acquired as well and were immediately placed on dry ice. After fixation, samples were wax embedded and cut with a microtome. Cut sections (5 μm) were stained with hematoxylin and eosin. For immunostaining, sections were subjected to immunodetection (horseradish peroxidase) or fluorescence detection.

γH2AX Immunostaining
Immunostaining was performed as previously described. This included antigen unmasking (10 mmol/L sodium citrate buffer), blocking, and permeabilization (10% normal goat serum in PBS with 0.1% Triton X-100), and subsequent incubation with anti-γH2AX (monoclonal, Millipore) at 4°C overnight. Signal detection was performed after incubation with biotin-conjugated goat antimouse secondary antibody (Thermo kit), diaminobenzidine (Sigma Aldrich), and H$_2$O$_2$ as a substrate. Counterstaining of the nuclei was performed with hematoxylin. Alternatively, microscopic signal detection was performed after incubation with antimouse fluorochrome-conjugated antibody (Invitrogen Alexa 488 Green). Counterstaining of the nuclei was performed with 4,6-diamidino-2-phenylindole.

Protein Extraction and Western Blotting
Protein lysation and Western blotting were conducted according to our standardized protocols. Used antibodies were anti-caspase 3, anti–poly ADP ribose polymerase (Cell Signaling), antitubulin (Sigma Aldrich), and horseradish peroxidase–conjugated secondary antibodies (GE Healthcare Life Sciences). Protein expression was visualized using enhanced chemiluminescence (ECL, Amersham Biosciences) and detected on films (ECL-Hyperfilm, Amersham Biosciences).

Statistical Analysis
This is largely a descriptive study. When applicable, as for instance in case of perfusion parameters and treatment volumes, results are summarized as mean±SD. In case of a skewed distribution, the median and the range are stated.

Results
General
Intact hearts were explanted from 4 (30–40 kg) pigs. $^{12}$C radiation was delivered to 3 hearts; the fourth heart served as a control and was not irradiated. The mean time of cold ischemia was 40±15 minutes. After rewarming, reperfusion was slowly initiated and 2 hearts required defibrillation with application of a 15 J shock. Mean perfusate temperature was 37±0°C. The mean coronary perfusion pressure was 115.75±12.55 mm Hg. In the perfusate, the mean pH during the course of the study was 7.35±0.05 at a mean bicarbonate concentration of 22.0±2.7 mmol/L with a mean base excess of −3.5±3.4 mmol/L. Mean $P_{CO_2}$ was 41.4±3.3 mm Hg, mean $P_{O_2}$ was 373.9±99.8 mm Hg. Mean lactate concentration was 1.5±1.2 mmol/L. The mean glucose concentration was 9.3±0.8 mmol/L. Mean heart endurance from reperfusion until the end of the experiment was 5.25±0.75 hours. The median time from reperfusion until start of $^{12}$C beam delivery was 2.2 hours (range, 1.8–3.5 hours).

Carbon Ion Beam Contouring and Treatment Planning Outcome
$^{12}$C beam contouring and treatment planning outcomes in 3 planes are depicted in Figure 1A to 1C. The mean target volume contour/clinical target volume was 1.0±0.3 cm$^3$. The mean planning target volume was 25.0±2.0 cm$^3$. Applied doses to the planning target volume were 70, 90, and 160 Gy, respectively, for the 3 hearts. The coverage of the clinical target volume was 100% in all cases. A $^{12}$C beam (10-mm full-width at half maximum in the isocenter) was scanned over an average of 15±1 raster-slices with energies between 216 and 311 MeV/u. An amount of 35,000 to 60,000 minimum particles/ions per point were used on the scanning grid (Figure 2).

Heart Surface Electrogram Recordings: Findings
All hearts remained in sinus rhythm after reperfusion and initial defibrillation. No heart required sustained pacing. In the heart which was irradiated with 70 Gy, no change in the atrioventricular interval could be observed during the course of the study and after delivery of the total dose. At 1 hour 45 minutes after irradiation, no change in atrioventricular conduction or antegrade Wenckebach period could be detected using decremental pacing.

The heart treated with 90 Gy showed diminished atrioventricular conduction with type II atrioventricular block before irradiation. During the course of the irradiation, this heart demonstrated progressive atrioventricular prolongation with a baseline atrioventricular interval of 120 ms versus an atrioventricular interval of 440 ms at the end of the procedure. The course of atrioventricular interval prolongation and its correlation to the delivered dose are depicted in Figure 3.
A third irradiated heart received a dose of 160 Gy to the planning target volume. Delivery of 130 Gy revealed atrioventricular interval alternans with 1 impulse being conducted with an atrioventricular interval of 130 ms, whereas the following atrial impulse was conducted at 80 ms (Figure 4B). The ventricular interval in this heart also alternated between 530 and 680 ms in a 1:1 atrioventricular conduction relationship. This rhythm continued at a cycle length of 770 ms with complete heart block after 160 Gy were applied (Figure 4C).

PET-CT Scanning

A PET-CT scan was performed for 2 hearts (treated with 90 and 160 Gy) within the critical timeframe of 30 minutes after the dose had been delivered. The PET-CT scan along with an overlay of the planned dose and measured β+-activity is depicted in Figure 5. In both cases, there was correlation between the actual deposited doses in the septum, measured as β+-activity, to the predicted activity based on the treatment plans.

Gross and Microscopic Pathology

No macroscopically visible damage could be observed in the Triangle of Koch and parts of the atrioventricular septum. Microscopic analysis of the tissue in the superior portion of the Triangle of Koch did not indicate signs for apoptosis, contraction band necrosis, or loss of cross striations. Hypereosinophilia seemed to be present (Figure 6).

γH2AX Immunostaining

Target regions showed strong positive response of the phosphorylated histone 2AX (γH2AX); Figure 7A), whereas in areas out of the irradiation field γH2AX signals were weak (Figure 7B). The beam entry channel through the left atrial appendage similarly showed a weaker, although positive signal. The unirradiated control heart did not show a positive reaction to γH2AX in any of the respective regions (Figure 7C).

Protein Analysis: Markers for Apoptosis

Western blot analysis of the irradiated tissue compared with the samples out of the beam field and samples of the control heart did not reveal higher content of apoptotic markers such as cleaved caspase 3 as well as cleaved Poly ADP ribose polymerase (PARP [poly ADP ribose polymerase]; Figure 8).

Discussion

Main Findings

This study is the first investigation on the feasibility of using a focused, pencil-beam-scanned 12C beam for ablation of myocardium for treatment of cardiac arrhythmias. Treatment delivery to the atrioventricular node of isolated beating hearts could be accurately performed and validated using noninvasive PET-CT imaging and immunostaining. Delivery did not cause acute arrhythmic ventricular events,
even when high doses of irradiation were applied. It seems that at high dosage an acute end point effect can be seen. This study demonstrates that external beam ablation of the atrioventricular node in beating hearts may be feasible using a single horizontal path of a scanned $^{12}$C beam. After defining acute beam effects in these ex situ studies, we will be moving directly to chronic studies, which are pivotal for further investigation of this energy source for arrhythmia elimination.

x-Ray Induced Lesions in Acute and Chronic Studies

In this study, we ablated the atrioventricular node in beating isolated hearts using cutting-edge highly precise accelerated and scanned $^{12}$C. Complete atrioventricular block has been described as a complication after mediastinal and thoracic x-ray irradiations. Autopsy findings showed that damage was largely mediated through vascular injury. The vascular endothelium of different organs has been shown to be susceptible to ionizing irradiation. Damage to the endothelial cells leads to reduced perfusion in specific regions of the heart, with relatively rapid function impairment. Microvascular damage precedes any histologically proven necrosis in the affected area, whereas late effects may be induced at lower doses by accelerated atherosclerosis. After application of x-rays, myocardial fibrosis has been seen after doses between 20 and 30 Gy, suggesting that a threshold higher than this might be required for focal tissue ablation. This is concordant with findings of recent x-ray beam studies for arrhythmia ablation.

In their study using 60 Gy of a focal $\beta$-source for cardiac tissue ablation, Pérez-Castellano et al described endothelial denudation, elastic lamina disruption, and pulmonary vein sleeve necrosis in the acute animals studied. It was shown that these alterations progressed to intimal hyperplasia and matured to transmural fibrotic lesions within a follow-up of ≈90 days.
Previous Studies Using Extracorporeal Energy Sources for Arrhythmia Ablation

This study demonstrated sufficient tissue penetration of accelerated ¹²C ions to create atrioventricular block in beating isolated hearts. This finding corroborates studies using other energy sources with less robust focusing from an extracorporeal access point.⁶,²⁵

Sharma et al⁵ showed feasibility of limiting cardiac x-ray–mediated radiation effects to intended targets. Applying x-rays with a sophisticated delivery technique, this group targeted different cardiac locations, thereby changing local tissue function. Focused application in this and other approaches, such as IMRT (Intensity Modulated Radiation Therapy), is achieved by the use of several different beam angles limiting doses to surrounding tissues. However, this implies increased low-dose exposure of a large volume of myocardium⁶ as well as x-ray–mediated beam entrance effects.

Rationale for Moving Toward High-Energy Charged Particles

Because of the limitations of previous x-ray–based studies, most notably the insufficient focusing capabilities of x-ray–based approaches, we used accelerated ¹²C ions in the present study. Accelerated ¹²C ions have unique radiophysical properties. In contrast to x-rays, they release their energy at a target point when coming to rest in the tissue (Bragg peak) and can be applied in an extremely focused fashion.⁸
In the present study, we used pencil-beam-scanning of the \(^{12}\text{C}\) beam, which is accurate within a range of millimeters in the x, y, and z dimensions. However, the beam focus size (full-width at half maximum) was 10 mm. Modern \(^{12}\text{C}\) centers are able to generate an even more focused beam with a full-width at half maximum at the beam entry site of 3.5 mm. A small focus size seems favorable for cardiac targets and is likely to be used in the future. Radiofrequency energy, which remains the current mainstay of ablation therapy, is focused to the degree of 5 to 8 mm, a margin that may result in complications such as pulmonary vein stenosis and atrial-oesophageal fistula formation if handled incautiously.\(^1\)

The mechanism by which \(^{12}\text{C}\) irradiation leads to tissue damage and lesion formation is still under investigation, but overall seems to be similar to x-ray beams and thus dependent on DNA damage as well as formation of reactive oxygen species.\(^{26,27}\) However, \(^{12}\text{C}\) leads to more complex damage, including DNA double-strand breaks.\(^8\) DNA double-strand breaks are visualized by the use of \(\gamma\text{H2AX}\) staining,\(^{28}\) demarking the rapid phosphorylation of the histone H2AX in the chromatin surrounding a DNA double-strand break. In this study, this mechanism proved to be visible within hours in cardiomyocytes as well, being present in the target areas. One possibility is that this process will result in increased delayed cardiomyocyte apoptosis, although this is yet to be proven. This finding also confirmed \(^{12}\text{C}\) beam dose deposition into the target area on a molecular level, which adds an important piece of information on myocardial lesion initiation with \(^{12}\text{C}\).

To date, \(^{12}\text{C}\) ions have rarely been applied for localized treatment of cardiac tissue with the exception of case reports on the treatment of cardiac sarcomas.\(^{29}\) Of note, Amino et al\(^{30}\) irradiated left ventricles of rabbits after healing of a myocardial infarction using a lower dosed (5–15 Gy), \(^{12}\text{C}\) ion beam. After follow-up, rather than impaired, they observed improved cardiac conduction. This was mediated through upregulation of Connexin-43, pointing toward not only destructive cellular effects but improvement in conduction after \(^{12}\text{C}\) ion irradiation, depending on dosing.\(^{31}\)

**End Points for Lesion Creation**

We are well aware that the full effects of ionizing radiation take months to develop. In the present study, we were aiming to elucidate any acute effect of high doses of \(^{12}\text{C}\) ions on cardiac conductivity that could potentially be used as an acute marker for lesion creation. Therefore, we elected to escalate the dose from 70 to 160 Gy to provoke acute effects on conduction, if present. These data suggest that delivery of high doses of

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**Figure 6.** Hematoxylin and eosin staining of irradiated and unirradiated cardiomyocytes. **A**, Hypereosinophilia was observed in the triangle of Koch in the field irradiated with 160 Gy. **B**, These changes seemed not to be present out of the irradiation field and within the triangle of Koch of the control heart. **C**, Magnification, ×100.

**Figure 7.** \(\gamma\text{H2AX}\) analysis in and out of the carbon ion beam irradiation field. Comparison of the \(\gamma\text{H2AX}\) reaction with peroxidase and fluorescence detection in a probe obtained from the Triangle of Koch in comparison with a probe outside of the beam field. **A**, Irradiation field of the atrioventricular node treated with 160 Gy of \(^{12}\text{C}\). **B**, Probes obtained from the right ventricle were located out of the irradiation field (OOF). **C**, Unirradiated control heart. **Left**, Peroxidase reaction; brown are \(\gamma\text{H2AX}\)-positive DNA nuclei. **Middle**, All DNA in the nuclei is stained blue with 4′,6-diamidino-2-phenylindole (DAPI). **Right**, Green quantitative counterstaining of \(\gamma\text{H2AX}\) in the DNA. +, a positive reaction; +/-, a weaker positive reaction; and −, a negative reaction. Probe was considered negative if no nucleus showed a reaction for \(\gamma\text{H2AX}\). Magnification, ×60.
allow cardiac 4-dimensional CT data registration for guidance of a truly motion-accommodating treatment planning. Third, there was some uncertainty in positioning of the isolated heart and its stability in the organ bath. Therefore, in this feasibility study, these issues prompted us to decide for a shoot-large approach using several security margins to guarantee that, if existent, an acute biological effect could be seen. In the following intact animal studies, the ablation area for atrioventricular node ablation will be downsized.

The methods using ex situ perfused heart preparation did not allow performance of a follow-up with monitoring of electric alteration and lesion maturation over time. We are well aware that the overall effects of ionizing radiation occur within the timeframe of months. Thus, lesion maturation, the exact doses as well as translation to long-term tissue effects need to be investigated chronically in follow-up studies. Therefore, transfer of these acute findings to chronic outcomes needs to be awaited, especially as doses after which electric findings occurred were high. Nevertheless, using the Langendorff preparation, we could first investigate acute $^{12}$C beam effects on atrioventricular conduction. Second, verify beam deposition using PET-CT. Third, elucidate the first step in the molecular lesion cascade using a scanned $^{12}$C beam.

**Clinical Implications and Other Potential Applications in Cardiology**

Catheter-free ablation may have tremendous clinical implications and could potentially overcome limitations encountered with current catheter ablation strategies of many cardiac arrhythmias. Among others, these include the limited tissue penetration of radiofrequency energy and the challenge to create a transmural lesion, the high recurrence rates in certain arrhythmias, thromboembolic events, and other interventional risks. We presented simulations in CT data that demonstrated accurate $^{12}$C beam delivery for atrial fibrillation and atrioventricular node ablation.$^{32-34}$ Reliable targeting was achieved by application of a margin, covering cardiac motion and with ion beam delivery at expiration.$^{35}$ Rescanning of ions over the target volume$^{36}$ ensured homogeneous dose application, despite contractile motion. We aim to implement techniques such as ECG-based ion beam gating, leading to a true 5-dimensional delivery.$^{37}$ Thus, using different beam delivery approaches, our vision is to create the armamentarium, to thoroughly design catheter-free–delivered myocardial lesions of different sizes and shapes at any cardiac location within the best timeframe of the cardiac and respiratory cycle, being a powerful treatment option for a variety of cardiac arrhythmias.

If chronic studies corroborate the finding of the present study, this technology could also be of use for other procedures performed in cardiology such as septal ablation in hypertrophic cardiomyopathy and various denervation procedures in all instances, offering a catheter-free, fast, and less operator-dependent treatment approach.

**Conclusions**

This study is the first in vivo investigation on the methods and feasibility of cardiac ablation for arrhythmia treatment using a scanned $^{12}$C beam. Dose deposition into the atrioventricular...
node could be successfully demonstrated, despite interplay through cardiac motion. Our data suggest that high doses of 
\(^{12}\)C radiation lead to acute electric tissue effects, mediated through alteration in cell biology from ionizing radiation. Optimal doses for cardiac tissue ablation using \(^{12}\)C and proton beams are to be investigated in intact animal studies. \(^{12}\)C and other particle beams may form the foundation for catheter-free ablation to create lesions, well designed in size and shape, at any given cardiac location and merit investigation in intact animals.

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**Disclosures**

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