The Potential Application of Heavy Ion Beams in the Treatment of Arrhythmia: The Role of Radiation-Induced Modulation of Connexin43 and the Sympathetic Nervous System

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Abstract

It has been known that heart disease—such as myocardial infarction (MI), cardiac hypertrophy, or heart failure—alters the molecular structure and function of the gap junction, which can lead to an abnormal heart rhythm. Radiation has been shown to modulate intercellular communication in the skin and lungs by increasing connexin43 (Cx43) expression. Understanding how Cx43 upregulation is induced in a diseased heart can help provide a new perspective to radiation therapy for arrhythmias.

In a recent study with rabbits after MI, carbon ions were accelerated to 290 MeV/u and extracted in the air; a biologically (cell kill) uniform 6-cm spread-out Bragg peak beam was generated, and beam tissue depth was set to 30 mm with energy degraders to the depth position. Targeted heavy ion irradiation (THIR) with 15 Gy to the left ventricle increased Cx43 expression, improved conductivity, decreased the spatial heterogeneity of repolarization, and reduced the vulnerability of rabbit hearts to ventricular arrhythmias after MI. In clinically normal rabbits, THIR with 10 Gy caused a significant dose-dependent increase of Cx43 protein and messenger RNA 2 weeks after irradiation. The left (irradiated) and right (nonirradiated) ventricles exhibited circumferential upregulation of Cx43 lasting for at least 1 year. There were no significant changes in electrocardiograms and echocardiograms, indicating no apparent injury for 1 year. A single exposure of 135 MeV/u THIR with 15 Gy to a dog heart attenuated vulnerability to ventricular arrhythmias after the induction of MI for at least 1 year through the modulation of Cx43 expression. This long-lasting remodeling effect on gap junctions may lay the groundwork to novel therapies against life-threatening ventricular arrhythmias in structural heart disease.

To date, there have been few investigations into the effects of carbon-ion irradiation on electrophysiological properties in the human heart. Patients with mediastinum cancer were investigated for 5 years after treatment that included irradiation to the heart, and investigators found that carbon-ion beam irradiation to the heart is not immediately cardiotoxic and demonstrates consistent signals of arrhythmia reduction. Its practical application in non–cancer treatment, such as in arrhythmia treatment, is highly anticipated.

Keywords: Carbon-ion; Connexin43; gap junction; arrhythmia treatment; cancer patients
Introduction

There are 450,000 sudden cardiac deaths in the United States per year, and ventricular tachycardia/ventricular fibrillation (VT/VF) is reported as the cause in around 70% to 80% of the cases. Recently, it has been demonstrated that atrial fibrillation is associated with an increased risk of cardiovascular events and sudden cardiac death [1]. An analysis of 5200 adults age 28 to 62 years participating in the Framingham Heart Study showed that the lifetime risk of early death due to sudden cardiac arrest is lower for women (1 in 30) than men (9 in 30) [2].

An implantable cardioverter defibrillator has been reported to be useful for preventing sudden deaths, but its frequent use lowers not only quality of life but also survival rates [3]. Myocardial damage from electric shocks is thought to deteriorate cardiac function, but its mechanism remains unknown. A combination therapy with antiarrhythmic agents and catheter ablation is effective in reducing the number of ICD implantations. Amiodarone is effective in patients with organic heart diseases, but continued use over time can reduce its effect or cause side effects. In contrast, catheter ablation may lead to a cure, but surgeons need advanced skills and knowledge to undertake this operation. Catheter ablation is often effective in patients with endocardial lesions but can be hard to apply in those with epicardial lesions. Therefore, developing a new antiarrhythmic therapy is an important endeavor that appeals to both scientific and societal interests.

Myocardial Gap Junction Protein and Arrhythmia Occurrence

Three microstructures interconnect cardiomyocytes: the gap junction, desmosome, and fascia adherens. The latter 2, the desmosomes and fascia adherens, are the atrioventricular nodes of the cytoskeleton and contractile protein, respectively. As mechanically linked sites between cells, they do not contribute to excitation and conduction. In contrast, a gap junction comprises aggregates of multiple connexons interwoven with the cell membrane lipid bilayers and connexons from 2 adjacent cells with a narrow gap (of about 20 Å) that bind to each other and form an intracellular channel with a central diameter of 1 to 2 nm. Small molecule ions (<1 kDa) passing through them initiate electrical and metabolic connections that result in gap junction intercellular communication. Each connexon is composed of 6 subunits (known as connexin, Cx) (Figure 1A) [4].

A Cx is a protein with 4 transmembrane domains and a long intracellular C-terminus [5]. The Cx gene family comprises multiple genes and, in humans, >20 types of isoforms have been identified [6]. Mammalian cardiomyocytes express 3 types of Cxs—Cx40, Cx43, and Cx45—with molecular weights of 40 kDa, 43 kDa, and 45 kDa, respectively. Of these, Cx40 is found primarily in the atrial muscle and is abundantly expressed in the intraventricular stimulation conduction system (His bundle, bundle branches, and Purkinje fiber); Cx43 is most commonly expressed in the atrial and ventricular muscles; and Cx45 is mainly expressed in the sinoatrial/atrioventricular nodes (Figure 1B) [7].

For normal heart function, coordination of excitation and contraction via the gap junction is indispensable [8]. The diseased heart—owing to myocardial infarction (MI), cardiac hypertrophy, heart failure, and so on—shows that there is a transformation of molecular structure or function remodeling in the gap junction plaque [9] and forms a substrate of VT/VF and atrial fibrillation. “Substrate” is defined as a physiological and/or structural change that is identified as a low potential region by electrophysiological study. In the past, several studies have attempted to restore the electrical connections of ventricular myocytes by increasing the amount of Cx43 protein, such as with activation of Cx43 using endothelin-1 and angiotensin II [10], thyroid hormone analogs [11], rotigaptide (ZP123) [12], or nitrofen and vitamin A [13]. In mouse experiments, transplanting Cx43-expressing myocytes has been shown to significantly decrease post-MI VT inducibility [14].

Antiarrhythmic Action Due to Heavy Ion Irradiation in Animal Experiments

Gap junctions are distributed not only in the heart but also in other organs and are responsible for various functions, such as cell adhesion, cytoskeleton formation, adjacent cell homeostasis, and cancer cell suppression [15,16]. In the presence of highly malignant cancer cells, gap junctions control signal transduction, contributing to tissue invasion and suppression of metastasis [17]. In the field of oncology, it has been known since the 1990s that x-ray irradiation of rat alveolar epithelium [18] and mice skin cells [19] elevates the Cx43 protein level. A study using human diploid fibroblast cultures showed that alpha-ray irradiation improved intercellular communication by increasing the Cx43 level [20]. Activation of the Cx43 promoter is said to be induced by the nuclear factor of activated T cells and activator protein-1 [21,22], and it has been demonstrated that Cx43 expression is dependent on the radiation dose [23].

We hypothesized that by focusing on the phenomenon that radiation promotes the differentiation induction of the Cx43 protein, Targeted heavy ion irradiation (THIR) to the diseased heart can lead to the recovery of reduced Cx43 expression. In
cancer treatment with particle beam therapy, the irradiated area is determined by manipulating the spread-out Bragg peak. Focusing on this phenomenon, in 1997, we launched a noninvasive arrhythmia-treatment study using heavy ion beams at the Heavy Ion Medical Accelerator in Chiba as a joint study with the National Institute for Radiological Science [24]. The THIR was performed using 290 MeV/u carbon beams and a 6-cm spread-out Bragg peak as used in cancer treatments. An irradiation field of $2^2 \times 3^2$ cm$^2$ was set up with a rabbit’s left ventricular free wall as the target. The depth from the pericordial skin surface to the anterior surface of the left ventricle was estimated to be 2 to 3 cm considering variations in cardiac motion and respiration.

Rabbits with nontransmural MI by microsphere injections received a single dose of 290 MeV/u carbon beams with 15 Gy to heart. After 2 weeks, a considerable increase in the expression of Cx43 was detected in the infarcted area by immunostaining, reverse transcription polymerase chain reaction, and western blot (Figure 2). Electrophysiological experiments in vivo showed improved spatial heterogeneity of action potential duration, improved conduction velocity, and reduced VT/VF inducibility (Figure 3). These results suggest that heavy ion beams improve the electric coupling of ventricular myocytes via Cx43 upregulation and result in antiarrhythmic action [25].

Figure 1. Gap junction and connexin (Cx). (A) The gap junction is formed by 2 opposing subunits called the hemichannels or Cxs, one contributed by each cell. Each Cx is composed of 6 subunits. (B) Mammalian cardiomyocytes express 3 types of connexins: Cx40, Cx43, and Cx45. Reproduced with permission from Jongsma HJ, Rook M. Morphology and electrophysiology of cardiac gap junction channels. In Jalife J, Zipes DP, eds: Cardiac Electrophysiology. From Cell to Bedside. 2nd ed. Philadelphia, PA: WB Saunders; 1995:115–126; Severs NJ, Bruce AF, Dupont E, Rothery S. Remodelling of gap junctions and connexin expression in diseased myocardium. Cardiovasc Res. 2008;80:9–19. Severs NJ. Pathophysiology of gap junctions in heart disease. J Cardiovasc Electrophysiol. 1994;5:462–475; and Martin PE, Evans WH. Incorporation of connexins into plasma membranes and gap junctions. Cardiovasc Res. 2004;62:378–387.
As a subsequent study, we examined the duration of time- and dose-dependent effects on Cx43 using normal rabbits without MI. Comparing doses of 5 Gy, 10 Gy, and 15 Gy showed that Cx43 expression was significantly elevated when the dose was more than 10 Gy; we found that Cx43 expression was significantly elevated with doses \( \geq 10 \text{ Gy} \), and the effect persisted for at least 1 year at 15 Gy (Figure 4). Neither cardiac contractility nor dilated capacity was detected by cardiac ultrasound examination for a year, nor was pathological degeneration of the myocardium observed [26]. Next, we performed experiments at RIKEN using beagle dogs with non-transmural MI who received 135 MeV/u carbon beams to 15 Gy. After 1 year, a signal-averaged electrocardiogram examination showed an improved late ventricular potential deterioration after infarction (Figure 5) and a lowered induction rate of VT/VF [27].

### Cardiac Sympathetic Denervation After Heavy Ion Irradiation

There is a strong relationship between arrhythmia and cardiac sympathetic nerve remodeling [28] in addition to gap junction remodeling. In the human heart, extrinsic sympathetic innervation is mediated via the cervical, stellate, and thoracic ganglia [29]. Anesthetic inhibition or surgical resection of the stellate ganglion reduces ventricular arrhythmias [30,31]. Iodine-123 metaiodobenzylguanidine (\(^{123}\text{I-MIBG}\)) imaging has been a useful tool in diagnosing sympathetic function and distribution and, thus, has been clinical used in patients with organic heart disease to evaluate the risk of ventricular arrhythmias [32].

In patients with atrial fibrillation, sympathetic fibers and parasympathetic fibers are densely distributed over the roof of the left atrium and pulmonary veins [33]. Animal studies of dogs with pacing-induced heart failure have demonstrated that left and right thoracic sympathetic ganglion ablation reduces the number of atrial tachycardia episodes compared with a control group without stellate ganglia ablation [34]. Modifying autonomic nerve response is key to treating arrhythmia [35].

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In our first experiments in 1997 using 290 MeV/u carbon beam with a high dose of 90 Gy for non-transmural MI rabbits [36], we aimed to examine the influence of heavy ion on the cardiac sympathetic nerve. Examination with 123I-MIBG autoradiography 1 month after exposure confirmed uniform sympathetic denervations that corresponded to the irradiated area of the left ventricle. In contrast, myocardial necrosis was not widespread across the irradiated region, and its contractility was not affected. These results were presented at the American Heart Association conference in 2000 as the world’s first preliminary study of heavy ion beam application for antiarrhythmic therapy. An electrophysiological test following irradiation

![Figure 3](image)

**Figure 3.** Induction of ventricular tachycardia/ventricular fibrillation (VT/VF). (A) Representative electrocardiogram recordings in control, myocardial infarction (MI), and MI plus targeted heavy ion irradiation (MI-THIR) (targeted heavy ion irradiation) rabbits. After 5 basic stimuli (S1) at a cycle length of 200 ms, triple extra stimuli (S2-S4) were applied at progressively shorter coupling intervals under norepinephrine infusion (0.1 μg/kg/min intravenous). (B) Activation time (AT) maps of 2 sequential beats during the VF and VT episodes in the MI and MI-THIR rabbits as presented in A. (C) Incidence of VT/VF elicited by the programmed stimulation; sustained VT (≥30 s); nonsustained VT (<30 s). In control and control-THIR rabbits, no VT/VF was induced. In MI rabbits, 2 VF and 2 VT (one sustained >30 s, the other non-sustained) were induced. In MI-THIR rabbits, only 1 non-sustained VT (NSVT) was induced. Activation patterns during the NSVT were much more homogeneous than those during VF documented in MI rabbits in B. Reproduced with permission from Amino M, Yoshioka K, Tanabe T, Tanaka E, Mori H, Furusawa Y, Zareba W, Yamazaki M, Nakagawa H, Honjo H, Yasui K, Kamiya K, Kodama I. Heavy ion radiation up-regulates connexin43 and ameliorates the arrhythmogenic substrates in rabbit hearts after myocardial infarction. *Cardiovasc Res.* 2006;72:412–421.

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**In Figure 3:**

- **A** shows representative electrocardiogram recordings from three conditions: Control, MI, and MI-THIR. Each condition demonstrates varying waveforms, indicating differences in cardiac response.
- **B** displays activation time (AT) maps of two sequential beats during VT/VF episodes in MI and MI-THIR rabbits. The maps illustrate activation patterns before and after irradiation, highlighting the impact on cardiac activity.
- **C** outlines the incidence of VT/VF elicited by programmed stimulation. It presents data for control, control-THIR, MI, and MI-THIR rabbits, showing the number of sustained and nonsustained VTs.

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**Table:**

| Condition            | NSVT | SVT | VF | Vulnerability |
|----------------------|------|-----|----|---------------|
| Control (n=5)        | 0    | 0   | 0  | 0/5           |
| Control-THIR (n=5)   | 0    | 0   | 0  | 0/5           |
| MI (n=5)             | 1    | 1   | 2  | 4/5           |
| MI-THIR (n=5)        | 1    | 0   | 0  | 1/5           |

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with heavy ion showed distinct suppression of VT/VF inducibility compared with the control [37]. Prior to the presentation, a 3-dimensional model of varying severity of the denervated region using \(^{125}\)I-MIBG autoradiography was successfully constructed, and it has been demonstrated that the instability of the myocardial action potential increases in an inhomogeneous denervated region [38]. Since the dose used in the study at that time, a single bolus of 90 Gy, was high, it is unclear whether the same effect will occur with lower radiation doses. The mechanism of cardiac sympathetic denervation due to radiation is also unclear.

The myocardium is radioresistant; therefore, the onset of new malignant tumors accompanying radioactive myocardial damage is rare. In contrast, late occurrence of MI due to coronary artery occlusion is known. Such side effects are primarily

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Figure 4. Immunohistochemistry, western blotting, and reverse transcription polymerase chain reaction. (A) Representative confocal images of connexin-43 (Cx43) immunolabeling in longitudinal sections of the left ventricular anterior wall. In the control, Cx43 formed clusters of punctuate immunofluorescence domains confined to well-organized intercalated disks running perpendicular to the longitudinal axis. Targeted heavy ion irradiation (THIR) resulted in a characteristic increase in immunopositive Cx43 not only at the intercalated disk regions but also at the lateral cell borders during the entire follow-up period for 1 year. (B) The proportion of total cell area occupied by Cx43 immunoreactive signals in the combined analysis of 25 rabbits (5 in each group). The radiation resulted in a significant increase in the immunopositive signals by 71% to 116% compared with the control group \(P < .05\). We estimated the proportion of Cx43 label at the lateral cell surface (LS) over the total label, including both at the LS and at the intercalated disk region (ID). The values (LS/ID + LS) after THIR (28.2% ± 10.9% at 2 weeks, 30.2% ± 15.7% at 3 months, 28.1% ± 11.6% at 6 months, and 18.9% ± 14.2% at 12 months) were all significantly larger than the controls (9.3% ± 6.3%; \(P < .05\)). (C and D) The amount of Cx43 protein estimated by western blotting and the level of Cx43 messenger RNA estimated by reverse transcription polymerase chain reaction were also increased after THIR throughout the entire follow-up period from 2 weeks to 12 months by 37% to 55% and by 24% to 59%, respectively, compared with the controls \(P < .05\). Reproduced with permission from Amino M, Yoshioka K, Fujibayashi D, Hashida T, Furusawa Y, Zareba W, Ikari Y, Tanaka E, Mori H, Inokuchi S, Kodama I, Tanabe T. Year-long upregulation of connexin43 in rabbit hearts by heavy ion irradiation. Am J Physiol Heart Circ Physiol. 2010;98:1014–1021.
attributable to x-rays, and long-term observation data for heavy ion beams are still insufficient. If a risk of complications increases alongside the antiarrhythmic effect, the benefits of particle beam may be canceled out. There is a need to evaluate the safety of heavy ion beams through long-term follow-up of patients.

**Future Directions With Clinical Application**

As a preliminary study of arrhythmia treatment with heavy ion beams, we examined the influence of heavy ions on impulse conduction to the myocardium, focusing on patients who underwent thoracic radiation therapy for mediastinal tumors. Eight patients were enrolled in a prospective study between April and December 2009 (2 men and 6 women; average age, 72.5 years). The total irradiation dose was 44 to 72 Gy (RBE), and the heart irradiation dose was 1.3 to 19.1 Gy (RBE). A high-resolution ambulatory electrocardiogram was performed before and after carbon-ion radiotherapy to evaluate arrhythmic
events, depolarization abnormality by late potentials, and autonomic nerve function by heart rate variability. The timing of examinations were within a week before radiotherapy and 1 month after the final planning of radiotherapy.

The results revealed that, before irradiation, supraventricular and ventricular arrhythmia (including premature atrial contraction, paroxysmal atrial fibrillation, atrial fibrillation, and premature ventricular contraction) was observed in 5 patients, and 4 patients improved after irradiation, whereas 1 remained unchanged (Figure 6). Depolarization abnormalities improved in 2 patients with respect to both atrial and ventricular late potential, and there were no cases of deterioration. Six patients who were irradiated to both sides of the stellate ganglion showed either a reduction of relative sympathetic tone or no deterioration by heart rate variability analysis. Total, low-frequency, and high-frequency power increased during the 24 hours after radiotherapy compared with that before radiotherapy, whereas low-frequency/high-frequency was relatively decreased. These results were similar in the analyses for both the day (8:00–21:00) and night (23:00–6:00) periods.

At the 5-year follow-up, 6 patients had died of cancer and 2 were alive, neither of whom had a history of hospitalization due to a cardiac event. As mentioned earlier, it is possible that carbon ion radiotherapy does not result in acute cardiotoxicity and results in antiarrhythmic effects caused by arrhythmia substrate or autonomic nerve modifications [39]. This study has several limitations. First, causality is difficult to establish in a relatively low number of patients with various arrhythmias; therefore, the
data are vulnerable to biases. Nevertheless, it was suggested that the antiarrhythmic action obtained as a secondary effect may be a silver lining for cancer patients suffering from arrhythmia.

When applying a heavy ion beam in arrhythmia treatment, accurate targeting can be expected, especially with the epicardial arrhythmia substrate, to take advantage of the Bragg peak characteristics. In the United States, the feasibility of in vivo atrioventricular node ablation was investigated in Langendorff-perfused porcine hearts using a scanned carbon beam [40]. In Europe, a technique of minimally invasive ablation from outside the body using a carbon ion was tested as an alternative therapy to catheter ablation in patients with atrial fibrillation [41]. Clinical trials in progress are also analyzing the application of particle beams against VT (Phase I/II Study, NCT02919618, ENCORE-VT). Although not a particle beam study, in a study of stereotactic irradiation with x-rays to the trunk for VT in humans, all 5 patients reported a significant suppressive effect [42]. Although unresolved problems remain, such as safety and the establishment of a minimum effective dose, the practical application of heavy ion beams in non–cancer treatment, such as in arrhythmia treatment, is highly anticipated.

ADDITIONAL INFORMATION AND DECLARATIONS

Mari Amino and Koichiro Yoshioka participated equally in the current study.

Conflicts of Interest: The authors declare no conflicts of interest associated with this manuscript.

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Additional Statements: The Animal Ethics Committee of Tokai University approved this study. All animal experiments complied with the Guide for the Care and Use of Laboratory Animals (8th edition, updated version, 2011). The investigation conformed to the principles outlined in the Declaration of Helsinki. The internal review board of each institution approved the study protocol, and all patients gave written informed consent before enrollment.

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