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ORIGINAL RESEARCH

Electrophysiological and Pathological Impact of Medium-Dose External Carbon Ion and Proton Beam Radiation on the Left Ventricle in an Animal Model

Mitsuru Takami, MD, PhD; Tetsuya Hara, MD, PhD; Tomoaki Okimoto, MD, PhD; Masaki Suga, MP; Koji Fukuzawa, MD, PhD; Kunihiko Kiuchi, MD, PhD; Hideya Suehiro, MD; Tomomi Akita, MD; Makoto Takemoto, MD; Toshihiro Nakamura, MD; Jun Sakai, MD; Atsusuke Yatomi, MD; Kazutaka Nakasone, MD; Yusuke Sonoda, MD; Kyoko Yamamoto, MD; Hiroyuki Takahara, MD; Ken-ichi Hirata, MD, PhD

BACKGROUND: Medium-dose (25 gray) x-ray radiation therapy has recently been performed on patients with refractory ventricular tachyarrhythmias. Unlike x-ray, carbon ion and proton beam radiation can deliver most of their energy to the target tissues. This study investigated the electrophysiological and pathological changes caused by medium-dose carbon ion and proton beam radiation in the left ventricle (LV).

METHODS AND RESULTS: External beam radiation in the whole LV was performed in 32 rabbits. A total of 9 rabbits were not irradiated (control). At the 3-month or 6-month follow-up, the animals underwent an open-chest electrophysiological study and were euthanized for histological analyses. No acute death occurred. Significant LV dysfunction was not seen. The surface ECG revealed a significant reduction in the P and QRS wave voltages in the radiation groups. The electrophysiological study showed that the local conduction times in each LV site were significantly longer and that the local LV bipolar voltages were significantly lower in the radiation groups than in the control rabbits. Histologically, apoptosis, fibrotic changes, and a decrease in the expression of the connexin 43 protein were seen in the LV myocardium. These changes were obvious at 3 months, and the effects were sustained 6 months after radiation. No histological changes were seen in the coronary artery and esophagus, but partial radiation pneumonitis was observed.

CONCLUSIONS: Medium-dose carbon ion and proton beam radiation in the whole LV resulted in a significant electrophysiological disturbance and pathological changes in the myocardium. Radiation of the arrhythmogenic substrate would modify the electrical status and potentially induce the antiarrhythmic effect.

Key Words: carbon ion beam ■ electrophysiology ■ proton beam ■ radiation ■ ventricular arrhythmia

Management of ventricular tachyarrhythmia with structural heart disease is still challenging. Recently, several clinical studies have shown the stereotactic body radiation therapy using 25 gray (Gy) x-ray markedly reduced the burden of ventricular tachycardia.[1,2] However, Gianni et al[3] reported on radiation therapy using 25 Gy x-ray in patients with scar-related ventricular tachycardia resulting in late recurrence of the arrhythmia after the radiation. The mechanism of the antiarrhythmic effect and the time course of 25 Gy radiation therapy in the left ventricle (LV) are poorly understood.

In addition, the side effects of radiation in the surrounding tissues of the heart are still problem.[2] Ionizing radiation is generally divided into electromagnetic waves (eg, x-rays, γ-rays) and particle beams (eg, proton,

Correspondence to: Mitsuru Takami, MD, PhD, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe, Hyogo, Japan 650-0017. E-mail: mitsuru_takamin@yahoo.co.jp

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CLINICAL PERSPECTIVE

What Is New?

- External beam radiation using medium-dose (25 gray equivalent) carbon ion and proton beam resulted in significant electrophysiological disturbance and pathological changes in the myocardium of the left ventricle.
- Most electrophysiological and pathological changes were obvious at 3 months after radiation and continued at the 6-month follow-up.
- A large radiation area in the present study resulted in pericardial effusion, precordial skin abnormalities, and partial radiation pneumonitis.

What Are the Clinical Implications?

- Medium-dose carbon ion and proton beam radiation can modify the arrhythmogenic substrate in patients with catheter ablation–resistant ventricular tachycardia or ventricular fibrillation.
- When using this treatment, the radiation area should be focused as narrowly as possible to minimize radiation exposure to the surrounding normal tissues.

Nonstandard Abbreviations and Acronyms

GyE	gray equivalent
HE	hematoxylin-eosin
RBE	relative biological effectiveness

carbon ion, helium ion, and neon ion). The x-ray deposits the maximum radiation energy close to the skin, after which the energy decreases gradually. After energy deposition in the target area, the x-ray beam continues beyond the target area, releasing energy and damaging the surrounding normal tissue. Unlike x-rays, particle beams can deliver most of their energy to the tissues in the distal region of their path, known as the Bragg peak,[4] and using this property, the radiation energy can be concentrated on the target area, which considerably decreases the energy deposition in the normal tissue. Thus, particle beam radiation therapy for cardiac arrhythmia may result in less damage to the surrounding tissues and organs than x-ray therapy. Among the particle beams, proton or carbon ion beam radiation has been used in clinical practice in patients with cancer and was available in 23 institutions in Japan in 2019.

Amino et al[5] reported that a low dose (15 Gy) of carbon ion beam radiation induced an increase in connexin 43 expression and improved conductivity in both normal hearts and hearts with myocardial infarction. In contrast, recent preclinical studies have shown that a higher dose of particle beam radiation (mainly >40 Gy) induces an electrical conduction block, apoptosis, and severe fibrosis in the targeted area of the heart.[6–9] These opposing results indicate that the effect of particle beam radiation of the myocardium might vary according to the radiation dose. However, there are limited data regarding the electrophysiological effect of medium-dose (25–35 Gy) radiation on the LV. Thus, the aim of the present study was to investigate the impact of medium-dose external carbon ion and proton beam radiation on electrophysiological and pathological changes and the time course in the LV myocardium in an animal model.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Overview

Figure 1A shows an overview of the present study. A total of 41 Japanese white rabbits weighing 2.7 to 3.1 kg were used. Of the rabbits, 32 were randomly assigned to 1 of 4 groups with 8 animals per group using block randomization; the radiation doses for the LV were 25 Gy equivalent (GyE) carbon ion beam (n=8), 35 GyE carbon ion beam (n=8), 25 GyE proton beam (n=8), and 35 GyE proton beam (n=8). A total of 9 rabbits did not receive cardiac irradiation (control group). At the end of the follow-up, all rabbits underwent an open-chest electrophysiological study and were then euthanized for histological analysis. All animal handling followed the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All procedures were approved by the Animal Experimentation Ethics Committee of Kobe University and Hyogo Ion Beam Medical Center.

Baseline Study, Computed Tomography Image

All rabbits underwent baseline ECG (model BJ607E; Nihon-Kohden Co. Ltd, Tokyo, Japan) and echocardiography (model HD11XE; Philips Inc.). They were sedated by intravenous injection of ketamine hydrochloride (15 mg/kg; Daiichi-Sankyo Co. Ltd., Tokyo, Japan) plus midazolam (1 mg/kg; Dormicum, Astellas Pharma Inc., Tokyo, Japan). Micro computed tomography scanners (Rigaku Co, Tokyo, Japan) were acquired for beam radiation treatment planning (Figure 1B).

Carbon Ion and Proton Beam Radiation

Treatment Planning

Treatment was planned using a computed tomography–based 3-dimensional treatment planning system

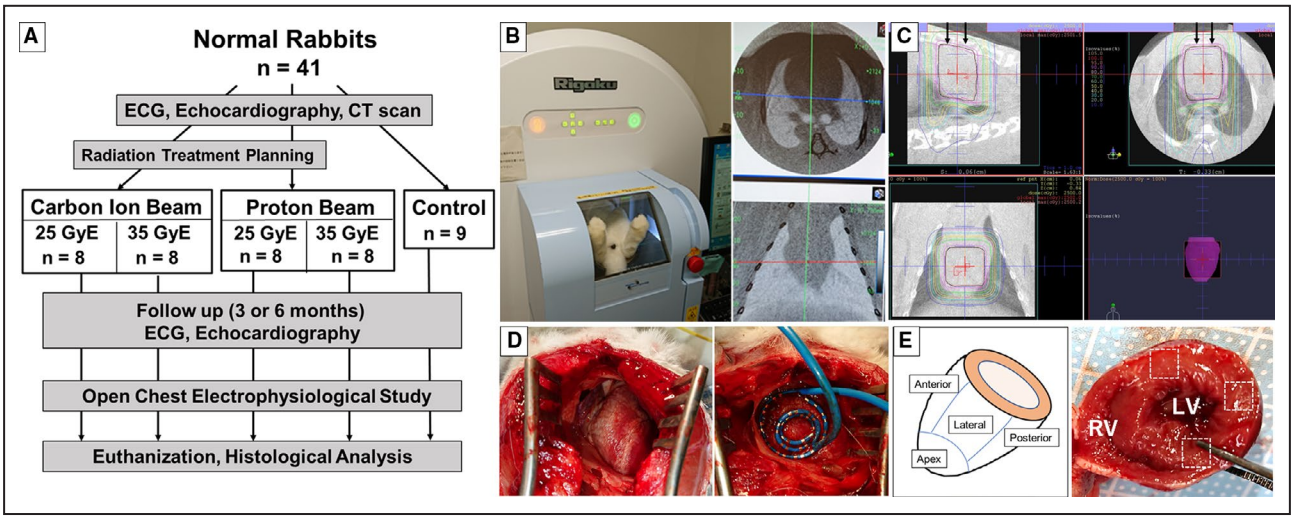


Figure 1. Study overview.
A, Protocol of the present study. **B**, Baseline CT images. **C**, Radiation treatment planning with 25 GyE carbon ion beam based on the CT. The beam radiation was performed through the anterior breast (the beam direction is indicated using black arrows). The black dotted line shows the isodose radiation area of the target dose. **D**, Open-chest electrophysiological study. Thoracotomy was performed, and the pericardium was removed. Two 20-pole circular mapping catheters (Inquiry Optima, Abbott) were placed on the epicardial LV parts (anterior, lateral, posterior, and apex). **E**, Four parts of the LV (anterior, lateral, posterior at papillary muscle level [white dotted square], and LV apex) were separated and histologically analyzed. CT indicates computed tomography; GyE, gray equivalent; LV, left ventricle; and RV, right ventricle.

(Xio-M [CMS and Mitsubishi Electric]) by a medical physicist at Hyogo Ion Beam Medical Center (Figure 1C). To avoid underdosing radiation in the LV, a radiation field was made using a 3×3 cm brass collimator. The spread-out Bragg peak length was 3 cm to cover most of the rabbits' LVs with isodose radiation.

Radiation Dose

When performing radiation therapy using particle beams, relative biological effectiveness (RBE) must be considered. RBE is the ratio of 1 type of ionizing radiation's biological effectiveness to another, given the same amount of absorbed energy. A carbon ion beam has a sharper Bragg peak and higher linear energy transfer ability, which results in a higher RBE. Doses of carbon ion and proton therapy are prescribed as Gy equivalent (GyE=Gy [RBE]) to reflect the fact that the dose was multiplied by an RBE value.[4] In the present study, an RBE value of 1.0 for x-ray (photon) was used as a reference, and the RBE values for carbon ion and proton beams were 2.8 and 1.1, respectively. The radiation dose for the LV was a 25 GyE carbon ion beam, a 35 GyE carbon ion beam, a 25 GyE proton beam, and a 35 GyE proton beam (Figure 1A).

Radiation Procedure

Hyogo Ion Beam Medical Center is the only institution in Japan that can use both carbon and proton beam radiation therapies. The rabbits were transferred to the research irradiation room and immobilized on the stand under intravenous sedation by ketamine hydrochloride plus midazolam. Echocardiography was performed just before radiation to identify the center of the LV. The entry site for the beam was marked on the precordial skin. During radiation, the rabbits were monitored by a high-resolution camera to confirm the location of the beam and the sedative status of the animals.

Follow-Up

All rabbits were followed up at the animal laboratory at Kobe University Hospital. The follow-up period was randomized to be either 3 months (carbon ion beam, n=8; proton beam, n=8) or 6 months (carbon ion beam, n=8; proton beam, n=8). All rabbits underwent regular ECG and echocardiography during the follow-up.

Open-Chest Electrophysiological Study

An open-chest electrophysiological study was conducted under general anesthesia (Figure 1D). The rabbits were sedated by intravenous injection of ketamine hydrochloride (15 mg/kg) plus midazolam (1 mg/kg). After intubation, the animals were ventilated on 1% to 3% isoflurane and monitored using surface ECG electrodes and SpO_2. Thoracotomy was performed, and the pericardium was removed. Two

20-pole circular mapping catheters (Inquiry Optima, Abbott) were placed on the epicardial parts of the LV (anterior, lateral, posterior, and apex). The epicardial conduction time and local voltages were then recorded during sinus rhythm. Conduction time was defined as the time between the local electrogram's deflection in the earliest activation site to its deflection in the last activation site in each part of the LV. The electrophysiological data were analyzed using Prucka CardioLab (GE Medical Systems Information Technologies). An arrhythmia induction test was performed. Single, double, and triple extra stimuli and burst pacing were performed through the electrode at the LV apex.

Histological Analysis

After the electrophysiological study, the rabbits were euthanized by an intravenous injection of sodium pentobarbital (150 mg/kg). The heart, lungs, trachea, and esophagus were removed, and the gross pathological findings were assessed. The LV was sectioned to a 3 mm thickness on the short axis (from the mitral annulus to the LV apex), and 4 parts of the LV (LV anterior, lateral, apex, and posterior at the level of the papillary muscle [Figure 1E]) were separated and processed to obtain paraffin-embedded sections, frozen sections, and sections for protein analysis. For paraffin embedding, the ventricular muscle was immersion fixed with a 10% neutral buffered formalin solution and embedded in paraffin. A total of 4 parts of the LV sections were stained with hematoxylin-eosin (HE) and Masson's trichrome. The area of fibrosis was quantified using ImageJ software. The area of fibrosis was presented as the fibrosis percentage (ie, the area of fibrosis divided by the total myocardial area). TUNEL (terminal deoxynucleotidal transferase–mediated biotin–deoxyuridine triphosphate nick-end labeling) staining was performed on the frozen sections to assess the apoptotic changes in the myocardium. To assess the gap junction function in the LV myocardium, western blotting was performed using antibodies directed against connexin 43 (anti-connexin 43, clone 4E6.2, MAB3067; Merck Millipore, Germany), and the densities of the connexin 43 bands were then quantified using an image analyzer. The densities were normalized using an actin signal.

The coronary artery was assessed for occlusion, stenosis, and injury. A total of 3 sections of the left anterior descending coronary artery were stained with HE.

The lung, esophagus, and trachea were immersion fixed with a 10% neutral buffered formalin solution and embedded in paraffin. A total of 3 parts of the lung adjacent to the heart and 3 parts outside the radiation area were obtained from the right and left lungs. Two sections of the esophagus adjacent to the heart were obtained. These sections were stained with HE.

Statistical Analysis

A previous study showed that the mean local conduction time of epicardial LV in myocardial infarction rabbits manifested an 8 ms increase compared with that of the control rabbits.[5] Based on the results of the study, the difference in the mean local conduction time of epicardial LV between the radiation group and the control group in the present study can be assumed to exhibit a 10 ms increase in light of the presumed effect of the radiation. For the analyses of the conduction time using *t* tests with 2-sided significance levels of 5% and 80% power, the required number of subjects was calculated to be 4 per group. To allow for 4 dropouts, the target sample size of this study was set to 8 per group.

Continuous variables were examined using the Shapiro-Wilk test for normality. For continuous variables of normal distribution, the data were presented as mean±SD. When the continuous variables had some evidence of nonnormal distribution, the data were presented as median (quartile). For data of normal distribution, the intergroup comparison (comparison of the effective refractory period of the LV, comparison of the area of fibrosis in the myocardium) was performed with a 1-way ANOVA test. For data of nonnormal distribution, multiple comparisons were performed using the Steel method (comparison of each radiation group to the control group) or the Steel-Dwass method. The *P* values were 2-sided. A *P* value of <0.05 was considered statistically significant. All statistics were calculated with R statistical software (version 2.13.0; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Events During the Follow-Up Period

Table shows the follow-up data after radiation. No acute death occurred after radiation, but 1 rabbit that underwent 25 GyE proton beam radiation died 2 months afterward. No significant decrease in LV ejection fractions and valvular abnormalities were observed in the rabbits. Mild to moderate serous pericardial effusion was observed in 3 of 16 (19%) rabbits in the carbon ion beam groups and 7 of 16 (44%) rabbits in the proton beam groups. Severe pericardial effusion or cardiac tamponade was not observed in any rabbit.

Surface ECG Changes

Figure 2 compares the surface ECG parameters of the control and radiation groups. The PQ intervals were

significantly longer in the 25 GyE proton beam groups than in the control group. The QT intervals were not significantly different between the control and radiation groups. The P and QRS wave voltages were significantly lower in the carbon ion and proton radiation groups than in the control group.

Electrophysiological Study Findings

An open-chest electrophysiological study was performed in 40 rabbits (9 control rabbits, 8 carbon ion beam 25 GyE rabbits, 8 carbon ion beam 35 GyE rabbits, 7 proton beam 25 GyE rabbits, and 8 proton beam 35 GyE rabbits). A mean of 51±9 epicardial electrical mapping points was obtained in each rabbit's heart.

Local Conduction Time in LV

Figure 3A shows a representative case of local conduction time in the LV anterior wall. The local conduction time was 9 ms in 1 of the control rabbits, and it was 23 ms in 1 of the rabbits in the 25 GyE carbon ion beam group. Figure 3B through 3E compares the local conduction times of the control and radiation rabbits in each part of the LV. Local conduction times were significantly longer in the radiation groups. Figure 4 compares the local bipolar voltages in each part of the LV, which were significantly lower in the radiation groups than in the control group.

Refractory Period and Inducibility of Ventricular Arrhythmias

An effective refractory period of the LV was 99±7 ms in the control group, 102±10 ms in the carbon ion beam group, and 120±17 ms in the proton beam group (P=0.003). No ventricular arrhythmias were induced in the control group or proton beam group, but ventricular fibrillation was induced in 1 rabbit in the 25 GyE carbon ion beam group.

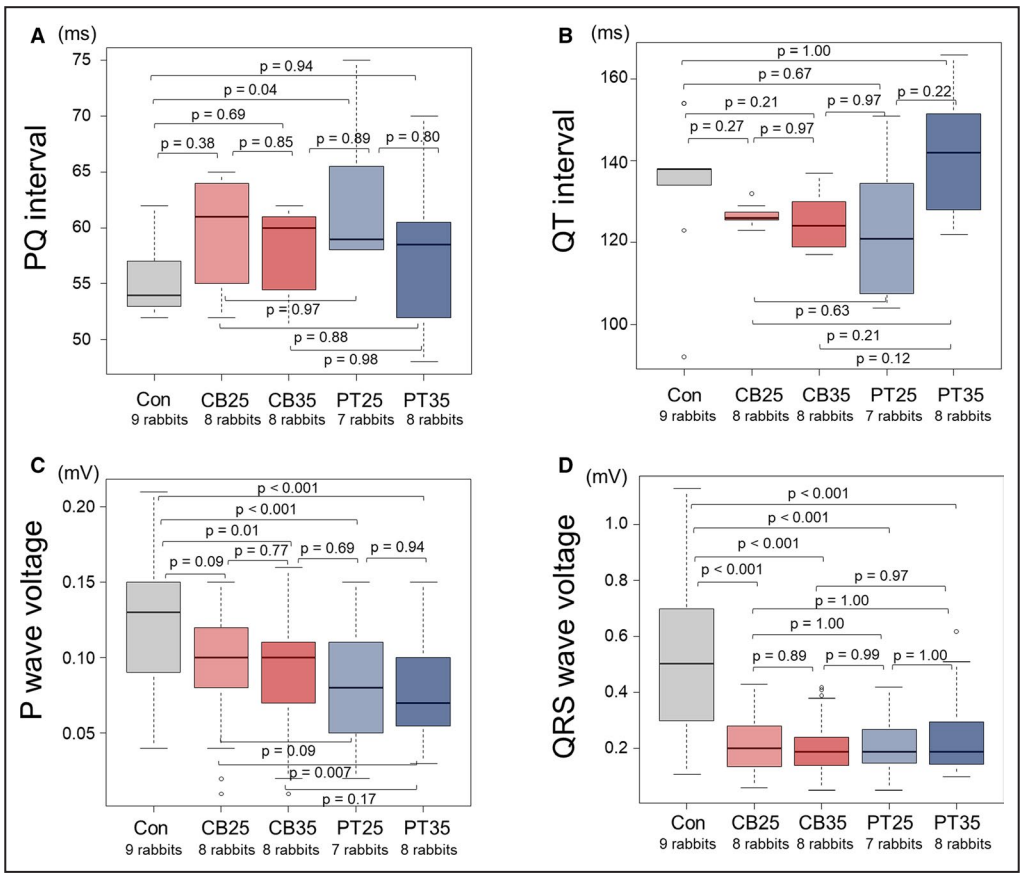


Figure 2. Surface ECG changes after radiation.
Surface ECG was performed in 40 rabbits (9 control rabbits, 8 carbon ion beam [25 GyE] rabbits, 8 carbon ion beam [35 GyE] rabbits, 7 proton beam [25 GyE] rabbits, and 8 proton beam [35 GyE] rabbits). **A**, PR interval. The PR interval was significantly longer in PT25 group than in the control group. **B**, QT interval. The QT interval was not significantly different between the control and radiation groups. **C** and **D**, P and QRS wave voltages. Both the P and QRS wave voltages were significantly lower in the carbon ion and proton beam radiation groups than in the control group. CB25 indicates carbon ion beam 25 GyE radiation; CB35, carbon ion beam 35 GyE radiation; Con, control; GyE, gray equivalent; PT25, proton beam 25 GyE radiation; and PT35, proton beam 35 GyE radiation.

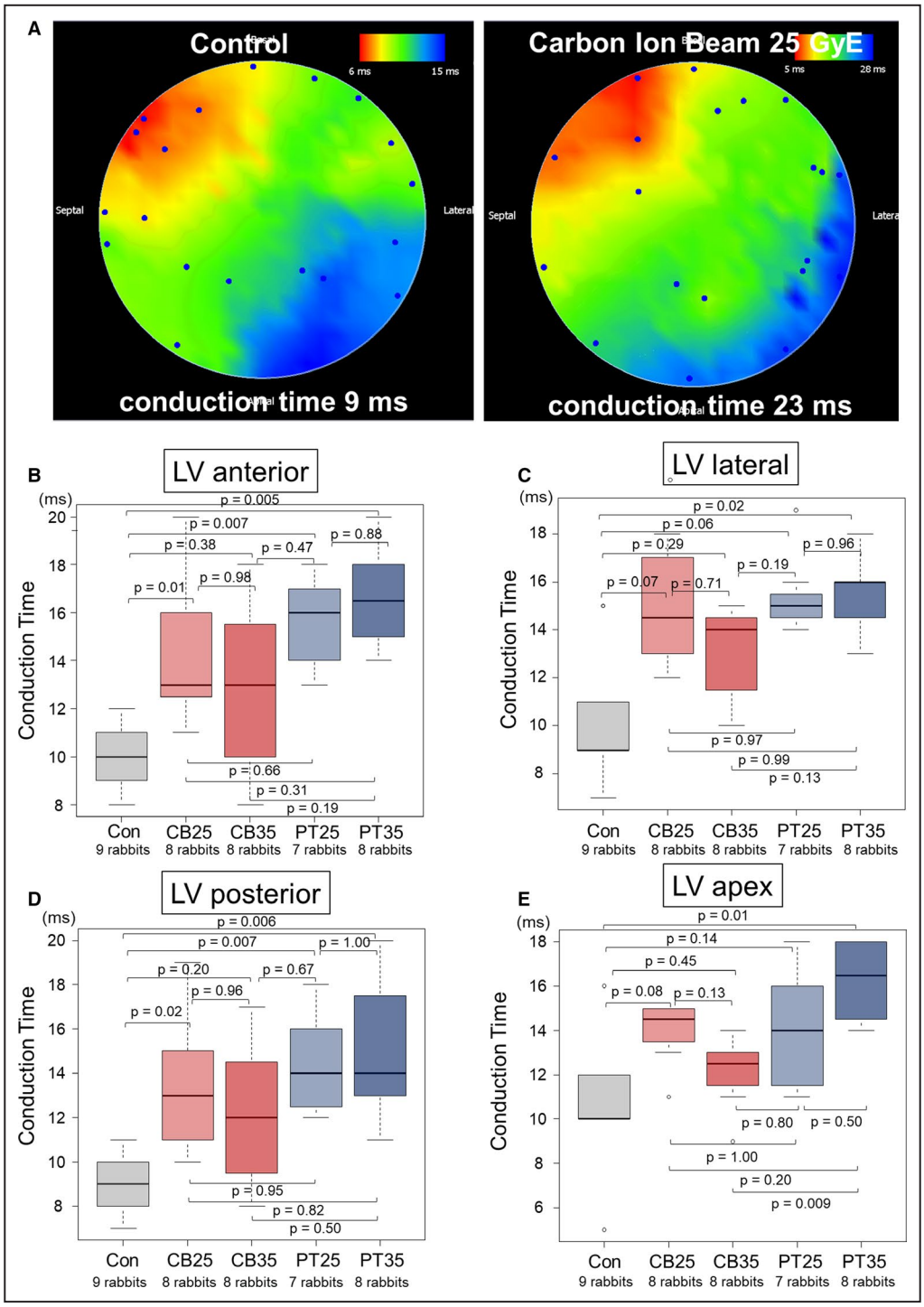


Figure 3. Local conduction time in the LV after radiation.
Local conduction time in each LV part was measured in 40 rabbits (9 control rabbits, 8 carbon ion beam [25 GyE] rabbits, 8 carbon ion beam [35 GyE] rabbits, 7 proton beam [25 GyE] rabbits, and 8 proton beam [35 GyE] rabbits). Representative conduction map in the LV anterior during sinus rhythm (**A**). Sinus conduction propagated from the septal to the lateral side (red, orange, yellow, green, and blue). The diameter of the circle was 10 mm. The conduction time in a control rabbit was 9 ms. However, the conduction time in a rabbit receiving the 25 GyE carbon ion beam was elongated to 23 ms. Comparisons of local conduction time between the control group and radiation groups in the (**B**) LV anterior, (**C**) LV lateral, (**D**) LV posterior, and (**E**) LV apex. CB25 indicates carbon ion beam 25 GyE radiation; CB35, carbon ion beam 35 GyE radiation; Con, control; GyE, gray equivalent; LV, left ventricle; PT25, proton beam 25 GyE radiation; and PT35, proton beam 35 GyE radiation.

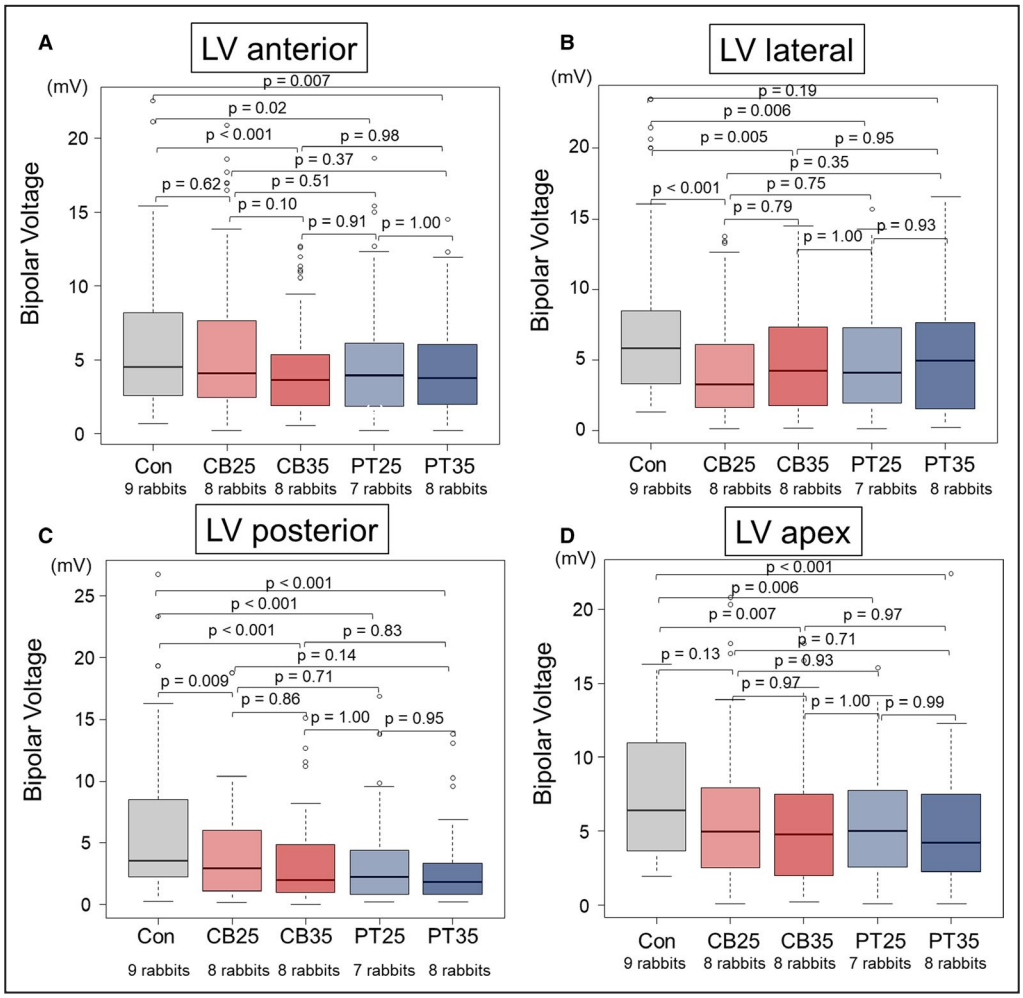


Figure 4. Local bipolar voltage in LV after radiation.
Local bipolar voltages in each LV part were mapped in 40 rabbits (9 control rabbits, 8 carbon ion beam [25 GyE] rabbits, 8 carbon ion beam [35 GyE] rabbits, 7 proton beam [25 GyE] rabbits, and 8 proton beam [35 GyE] rabbits). Comparison of local bipolar voltages between the control group and radiation groups in the (**A**) LV anterior, (**B**) LV lateral, (**C**) LV posterior, and (**D**) LV apex. Local bipolar voltages were significantly lower in both the carbon ion and proton beam radiation groups than in the control group. CB25 indicates carbon ion beam 25 GyE radiation; CB35, carbon ion beam 35 GyE radiation; Con, control group; GyE, gray equivalent; LV, left ventricle; PT25, proton beam 25 GyE radiation; and PT35, proton beam 35 GyE radiation.

Time Course Changes

Figure 5 shows the time course changes of local conduction time and bipolar voltage at 3 and 6 months after radiation. Local conduction times in the carbon ion beam radiation group were significantly longer than in the control group at the 6-month follow-up. Local conduction times in the proton beam radiation group were significantly longer than the control group at 3 months, but they were not significantly different between the 3-month and 6-month follow-ups. The local bipolar voltages in the LV anterior and posterior in both the carbon ion and proton beam radiation groups were significantly decreased at the 3-month follow-up, but they were not significantly different between the 3-month and 6-month follow-ups.

Histological Changes in LV

HE and Masson Trichrome Staining

Figure 6 shows the LV myocardial stained by HE and Masson trichrome. The atrophic changes in myocytes and the fragmentation of muscle bundles were stronger in the 35 GyE radiation rabbits than in the 25 GyE radiation rabbits in both the carbon ion and proton beam groups (Figure 6A). Masson trichrome staining showed an increase in interstitial fibrosis in both the carbon ion and proton beam radiation groups (Figure 6B). The area of fibrosis in the anterior LV myocardium was 0.7±0.6% in the control group (n=9), 12.9±1.7% in the 25 GyE carbon ion group (n=4), 15.7±2.2% in the 35 GyE carbon ion group

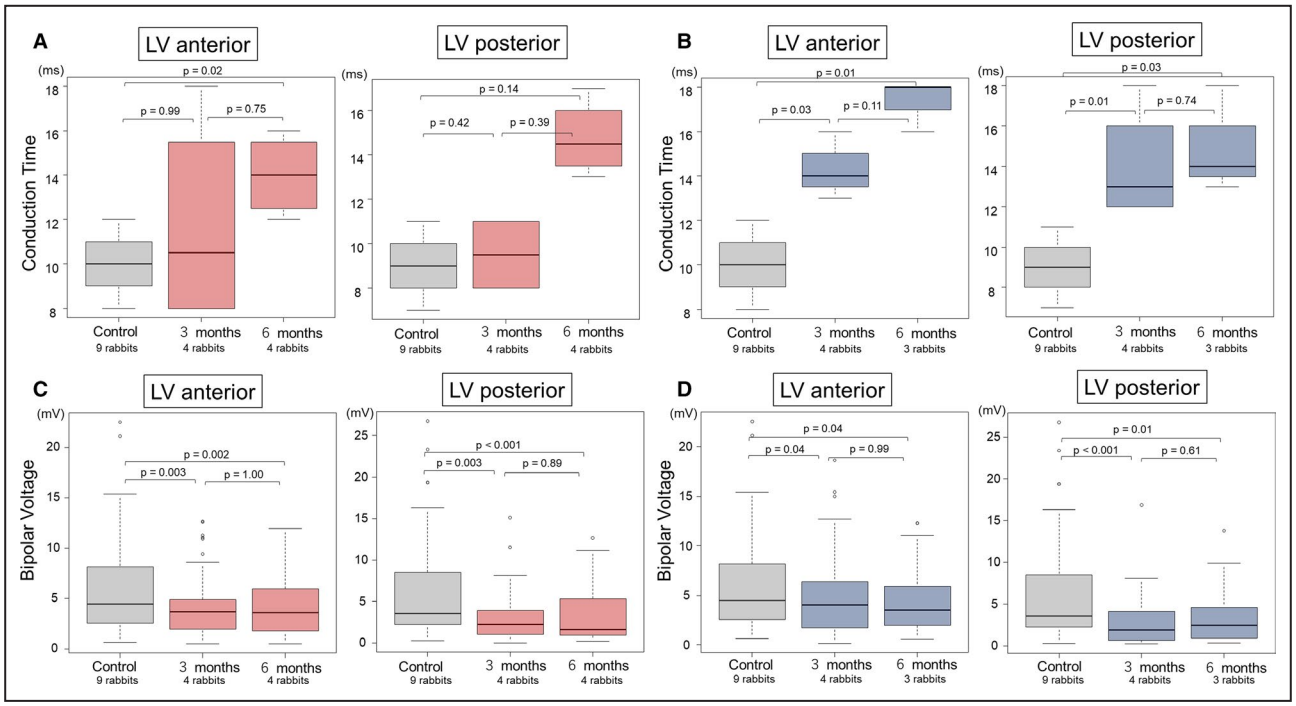


Figure 5. Time course changes of local conduction time and bipolar voltages.
A, Comparison of local conduction time between control and carbon ion beam radiation groups at 3 and 6 months after radiation. **B**, Comparison of local conduction time between control and proton beam radiation groups at 3 and 6 months after radiation. **C**, Comparison of local bipolar voltages between control and carbon ion beam radiation groups at 3 and 6 months after radiation. **D**, Comparison of local bipolar voltages between control and proton beam radiation groups at 3 and 6 months after radiation. LV indicates left ventricle.

(n=4), 13.7±4.0% in the 25 GyE proton beam group (n=4), and 14.3±2.2% in the 35 GyE proton beam group (n=4) (P<0.001).

Myocyte Apoptosis

Figure 6C shows myocyte apoptosis at 3 and 6 months after radiation, as evaluated by TUNEL staining. TUNEL-positive cells were not detected in the tissues of the control group. TUNEL-positive cells were seen in the LV myocardial tissues of the radiation groups both at 3 and 6 months after radiation.

Connexin 43 Protein Expression

Connexin 43 protein amounts were significantly lower in both the carbon ion and proton beam groups than in the control group (Figure 7A). Figure 7B and 7C demonstrate the time course of connexin 43 protein expression after radiation. The amount of connexin 43 protein significantly decreased at 3 months after radiation; however, it was not significantly different at the 3-month and 6-month follow-ups.

Effect on Other Tissues and Organs

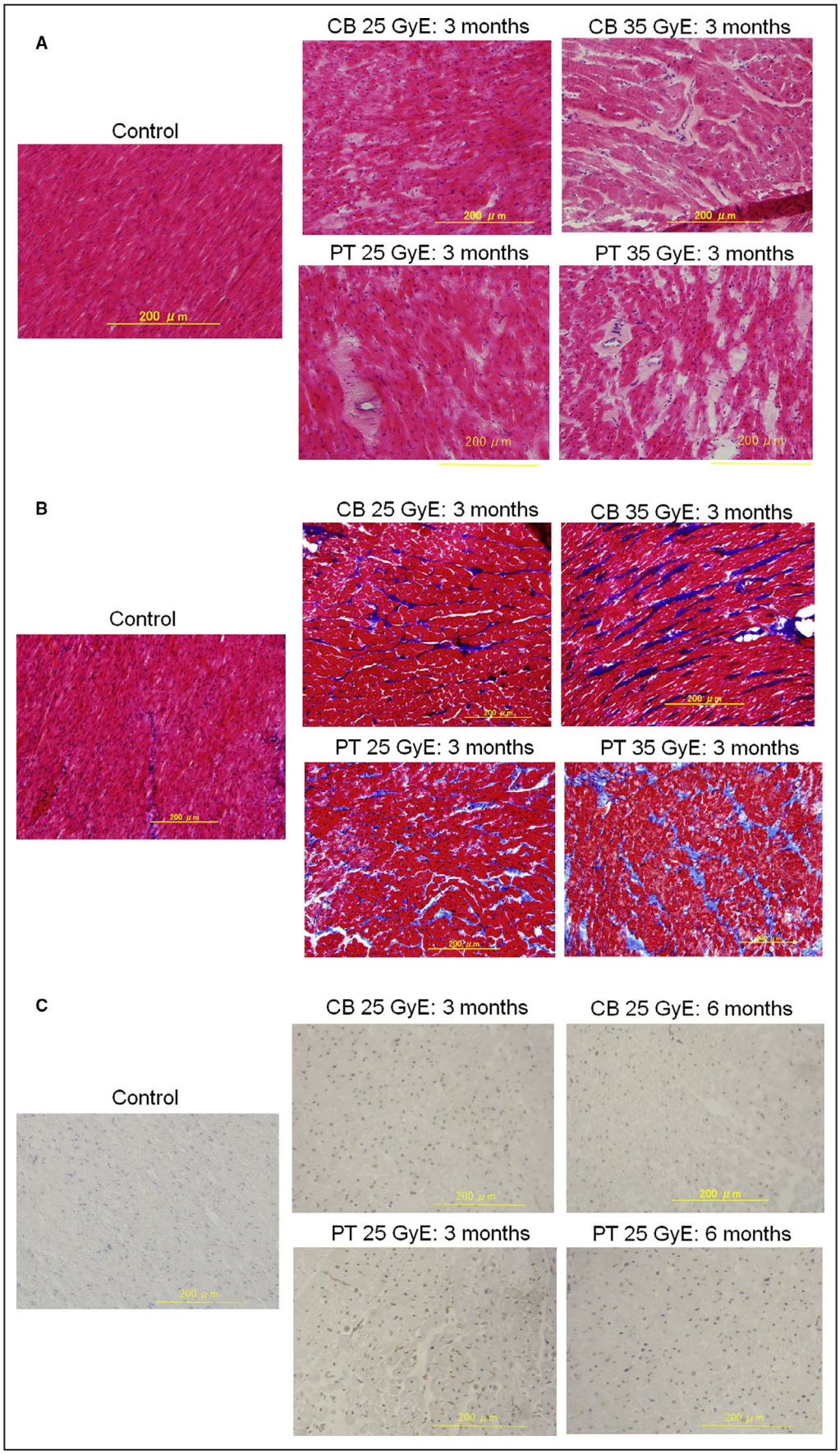
Skin

Precordial skin abnormalities, such as hair loss, redness, and erosion, were observed in 6 of 16 (38%) rabbits in the carbon ion beam group and in 16 of 16 (100%) rabbits in the proton beam group. These abnormalities were observed 1 month after radiation, but they improved during the follow-up period in most of the rabbits.

Coronary Artery

Macroscopically, no occlusion, stenosis, or injury was observed in the coronary artery of any rabbits. Figure 8A through 8C show a microscopic view of

Figure 6. Histological changes in LV myocytes after radiation.
A, HE staining of LV anterior myocytes. Atrophy and degeneration myocytes and fragmentation of muscle bundles were observed in carbon ion and proton beam radiation groups at 3 months after radiation. These changes were stronger after 35 GyE carbon and proton beam radiation than with 25 GyE beam radiation. **B**, Masson trichrome staining of the LV anterior myocardium showed increased interstitial fibrosis in carbon ion and proton beam radiation groups at 3 months after radiation. **C**, Apoptotic changes occurred in the LV anterior myocardium after 25 GyE radiation. In the radiation groups, apoptotic myocardial cells (blown color) were stained with TUNEL. Bar=200 μm. CB indicates carbon ion beam; GyE, gray equivalent; PT, proton beam; and TUNEL, terminal deoxynucleotidal transferase–mediated biotin–deoxyuridine triphosphate nick-end labeling.


A
Control
CB 25 GyE: 3 months
CB 35 GyE: 3 months
PT 25 GyE: 3 months
PT 35 GyE: 3 months
200 μm
B
Control
CB 25 GyE: 3 months
CB 35 GyE: 3 months
PT 25 GyE: 3 months
PT 35 GyE: 3 months
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C
Control
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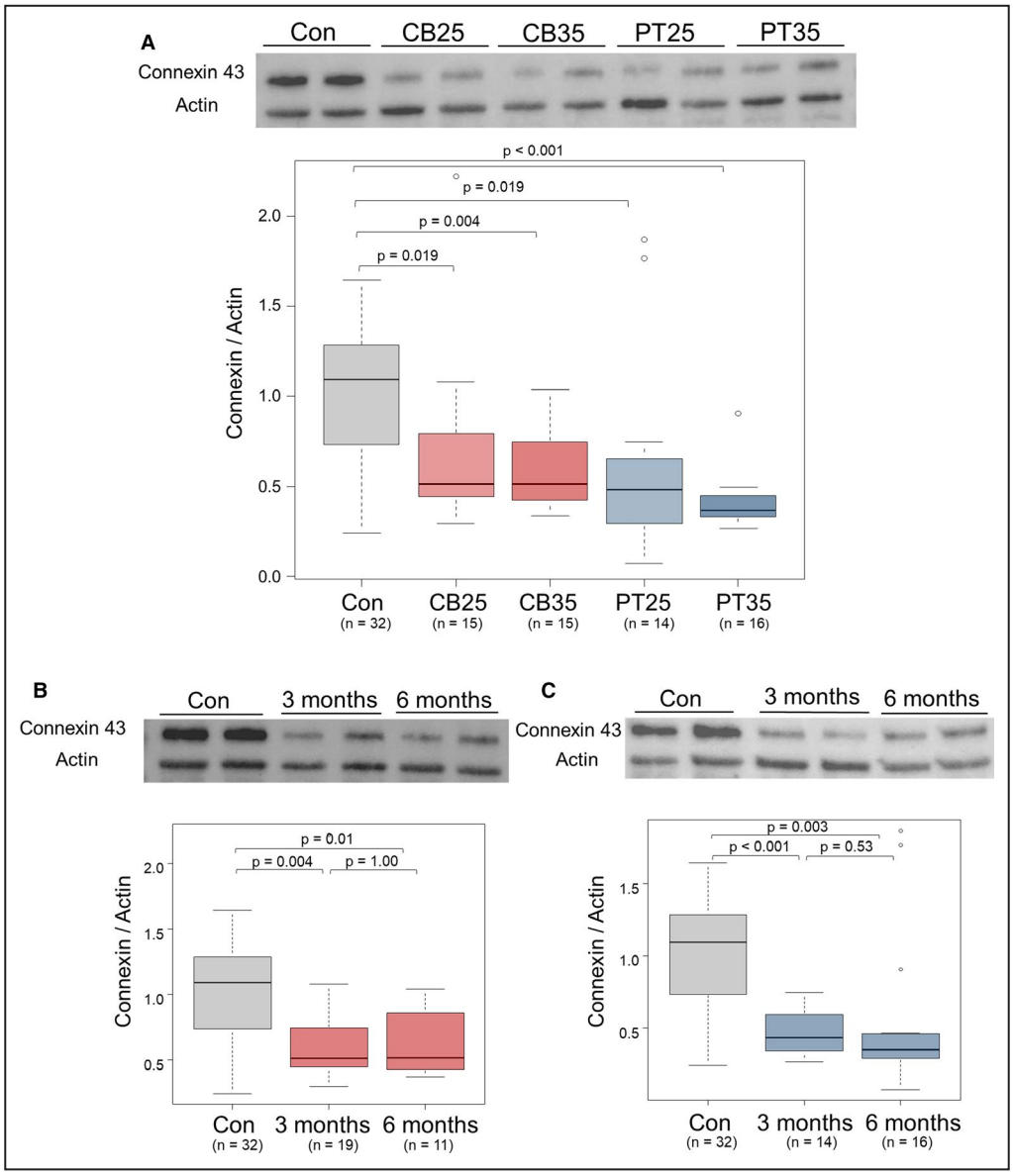

Figure 7. Connexin 43 protein expression in the LV after radiation.
A, Western blot for the connexin 43 protein in the LV anterior (control, n=32; carbon ion beam 25 GyE, n=15; carbon ion beam 35 GyE, n=15; proton beam 25 GyE, n=14; proton beam 35 GyE, n=16). Compared with the control group, the expression levels of the connexin 43 protein were significantly lower in the carbon ion and proton beam radiation groups (control vs each group, all $P<0.05$). **B**, Time course of connexin 43 protein expression after carbon ion beam radiation. Connexin 43 protein expression significantly decreased at 3 months after radiation (control vs 3 months, control vs 6 months, both $P<0.05$). The level of connexin 43 was not significantly different between the 3-month and 6-month follow-ups. **C**, Time course of connexin 43 protein expression after proton beam radiation. Connexin 43 protein expression significantly decreased at 3 months after radiation (control vs 3 months, control vs 6 months, both $P<0.05$). The level of connexin 43 was not significantly different between the 3-month and 6-month follow-ups. CB25 indicates carbon ion beam 25 GyE radiation; CB35, carbon ion beam 35 GyE radiation; Con, control group; GyE, gray equivalent; PT25, proton ion beam 25 GyE radiation; and PT35, proton ion beam 35 GyE radiation.

cross-sections of the left anterior descending coronary artery in the control and radiation groups. There was no evidence of atherosclerotic changes or stenosis in the left anterior descending coronary artery of radiation groups.

Lung

Figure 8D through 8H show gross and microscopic pictures of the lung. Compared with lung tissue in the control group (Figure 8D), no specific changes were observed in tissue from the lateral part of the lung

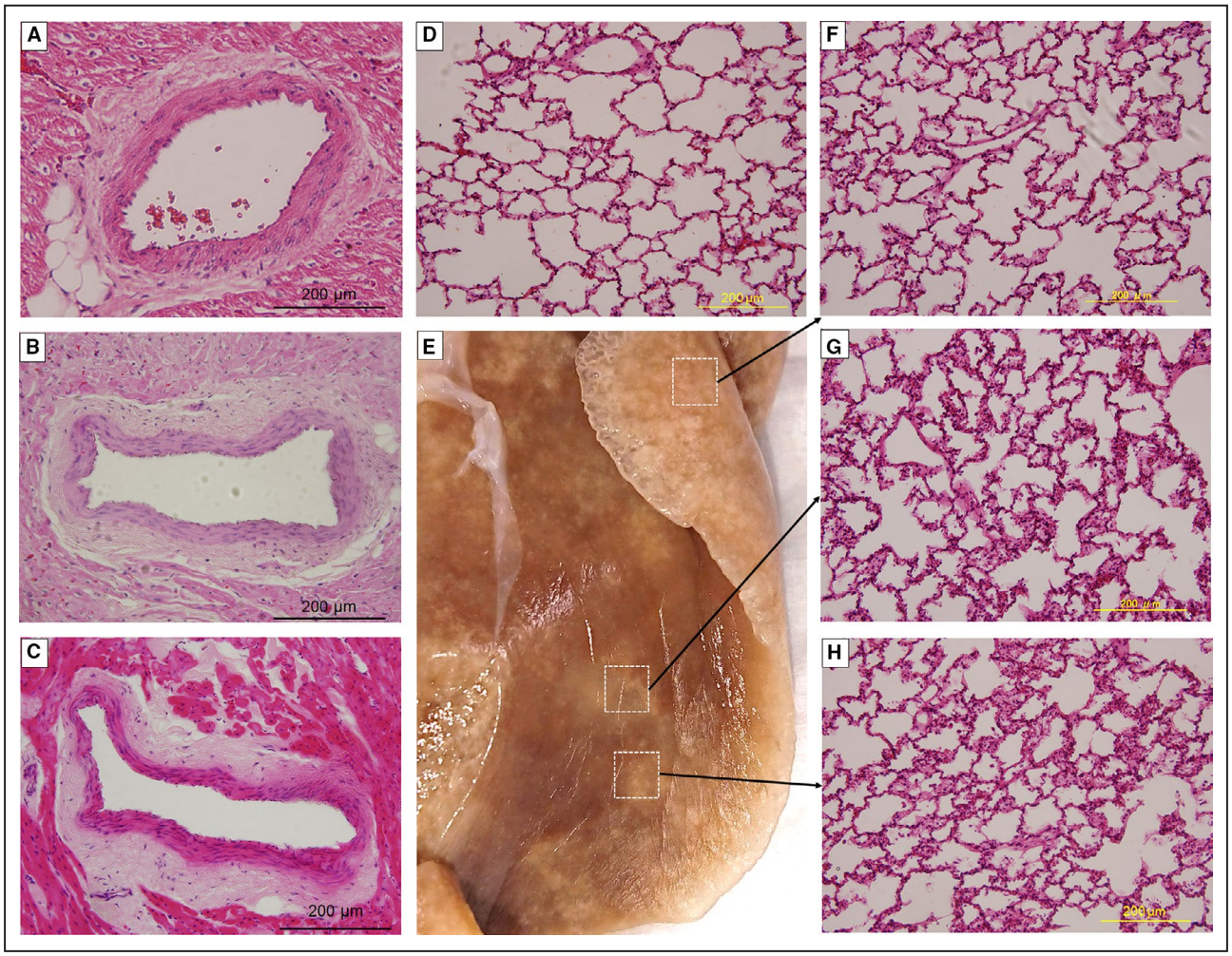

Figure 8. Radiation effect on the coronary artery and lung.
Cross-section of the left anterior descending coronary artery with hematoxylin-eosin staining (**A**) in a control rabbit, (**B**) in a 25 GyE carbon ion beam radiation rabbit, and (**C**) in a 25 GyE proton beam radiation rabbit. No evidence of atherosclerotic changes or stenosis in the coronary artery was observed in the radiation rabbits. **D**, Lung tissue with hematoxylin-eosin staining in a control rabbit. **E**, Macroscopic findings of the left lung in a 25 GyE carbon ion beam radiation rabbit. A total of 3 tissue sections were obtained from the lung (white dotted square). **F**, Lung tissue at a distance from the heart showed no significant changes compared with the lung tissue in a control rabbit. **G** and **H**, Lung tissues within the irradiation area adjacent to the heart. These tissues showed increased inflammatory cells and mild septal thickening, which indicated radiation pneumonitis. Bar=200 μm. GyE indicates gray equivalent.

(away from the heart) in a radiation rabbit (Figure 8F). Lung tissues from the area adjacent to the heart revealed an increase in inflammatory cells and mild septal thickening (Figure 8G and 8H). These changes were observed in 4 of 16 (25%) rabbits in the carbon iron beam group and in 4 of 16 (25%) rabbits in the proton beam group.

Esophagus and Trachea

No macroscopic or microscopic damage was observed in either the esophagus or trachea in any radiation rabbits.

DISCUSSION

This is the first study to examine both electrophysiological and pathological changes in the LV following a medium dose of carbon ion or proton beam radiation. The main findings of this study are as follows: (1) medium-dose (25 GyE) carbon ion and proton beam radiation induced significant conductive disturbance and decreased electrical activity in the LV myocardium; (2) pathological analysis revealed myocardial apoptosis, decreased expression of connexin 43 protein, and fibrotic changes; (3) most of the electrophysiological and pathological changes were obvious at 3 months

Table. Follow-Up Data

	Control (n=9)	Carbon Ion, 25 GyE (n=8)	Carbon Ion, 35 GyE (n=8)	Proton, 25 GyE (n=8)	Proton, 35 GyE (n=8)
Acute death	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chronic death	0 (0)	0 (0)	0 (0)	1 (13)	0 (0)
Weight (baseline), kg	2.9±0.1	2.8±0.1	2.9±0.1	2.9±0.1	2.9±0.1
Weight (end of follow-up), kg	3.1±0.1	3.2±0.1	3.2±0.1	3.1±0.1	3.1±0.1
Weight change percentage from baseline to follow-up, %	7±3	12±7	10±6	7±5	5±4
LVEF (baseline), %	68±2	68±2	68±2	68±1	69±3
LVEF (end of follow-up), %	67±2	67±2	66±2	67±2	66±4
Pericardial effusion	0 (0)	1 (13)	2 (25)	4 (50)	3 (34)

Data are shown as number (percentage) or mean±SD. GyE indicates gray equivalent; and LVEF, left ventricle ejection fraction.

after radiation and continued at the 6-month follow-up; and (4) a large radiation area resulted in pericardial effusion, precordial skin abnormalities, and partial radiation pneumonitis.

Effect of a Medium Radiation Dose on the Myocardium

Low-dose (15 Gy) carbon ion beam radiation has been reported to increase the expression of connexin 43 and improve the conductivity of the LV.[5] We speculated that a dose of ≈25 GyE carbon ion and proton beam radiation would be a turning point to damage the myocardium. Ionizing radiation damaged myocardial cells by breaking the DNA structure (double-strand DNA breaks) and generating reactive oxygen species,[4] which would induce LV myocyte apoptosis and mild fibrotic changes. Considering the results of previous studies using higher doses of carbon ion and proton beam radiation,[6–9] the radiation damage in the myocardium would be stronger with a radiation dose >25 GyE. The connexin 43 protein has been reported to be highly sensitive to ionizing radiation and other environmental stresses.[10] Contrary to the promotive effect of a 15 Gy radiation dose,[5] a radiation dose >25 Gy would deteriorate the expression of the connexin 43 protein and decrease the number of myocytes by inducing apoptosis. Consequently, the total amount of connexin 43 protein would decrease in the radiation rabbits who received higher doses, which elongate the local conduction time in each LV part.

Time Course of Radiation Effect in Myocardium

Muscle and nerve tissues, which no longer undergo cell division at the adult stage, are known to have low radiosensitivity (resistant to radiation).[11] Proton beam radiation in swine hearts with myocardial infarction demonstrated no radiation effect in the LV myocardium, as evaluated by magnetic resonance imaging at 4 weeks after radiation.[8] By 8 to 12 weeks after radiation, hypo-enhancement changes in the myocardial infarction site were observed. The present study also showed that most of electrical and pathological changes are not significantly different between the 3-month and 6-month follow-ups. These findings suggested that the pathological lesion formed in the myocardium by radiation would become mature by ≈3 months after radiation. Amino et al[5] reported that an improved conduction velocity of the LV was observed at 2 weeks after radiation. The antiarrhythmic effect of cardiac radioablation using 25 Gy x-ray happened within the first 6 weeks after treatment in most patients.[1,2] It is possible that electrophysiological changes would precede pathological changes after radiation. Further studies are needed to understand electrophysiological changes in the very early stages after medium doses of radiation.

Radiation Effects on Surrounding Tissues and Organs

The radiation area used in the present study was intentionally large for the animals' size to avoid underdosing radiation for the whole LV. This study may therefore also reveal the impact of radiation on surrounding tissues and organs. Any skin abnormalities would be caused by the anatomy of the rabbits. The particle beams entered the precordium, which is close to the heart in rabbits. A moderate radiation dose in the precordium would therefore cause hair loss, redness, and erosion. The pericardial effusion and partial radiation pneumonitis presented in this study have also been reported in patients who underwent 25 Gy x-ray radiation in the heart.[2] The radiation pneumonitis presented in this study was observed in the area of the lung adjacent to the heart. Ionizing radiation induces free radicals and DNA damage of the lung, and the damaged lung cells release cytokines, which attract inflammatory cells to the alveoli and pulmonary interstitium, causing this complication.[12]

Differences Between Carbon Ion and Proton Beam Radiation

The radiation doses were adjusted by taking into account the different biological effectiveness of the carbon ion and the proton beam. Thus, similar electrophysiological and histological effects in the LV myocardium were observed between the carbon ion and the proton beam radiation. Skin abnormalities at the radiation entry site were more frequently observed in proton beam radiation. In the energy deposition, the carbon ion beam had a sharper Bragg peak than the proton beam. Based on a preclinical biological assessment conducted at the Hyogo Ion Beam Medical Center, the proton beam showed a tendency for higher irradiation dose at the skin surface.[13] This tendency was also observed in the collected data in the current study.

Advantages of Particle Beam Radiation and Their Clinical Use

Particle beam radiation can narrow the field of irradiation when a high dose is given to the target area. In cancer treatment, the lifetime attributable risk of radiation-induced secondary cancer was significantly lower when using proton beam therapy than when using intensity-modulated x-ray therapy in pediatric patients.[14] The focus of the present study was not to investigate the accuracy of particle beam radiation of a target area being moved by cardiac beating and respiratory motion. However, the patient's motion management and target motion–tracking technology have been dramatically advanced in cancer treatment.[15] These technologies are applicable to radiation therapy in the heart.

In the present study, medium-dose carbon ion and proton beam radiation did not induce a complete conduction block, severe fibrosis, or scar formation in the normal heart. However, the arrhythmogenic substrate usually consists of surviving myocytes interspersed with fibrous and fatty tissues.[16] The sensitivity of damaged myocytes to radiation would be higher than that of normal myocytes. In addition, radiation's impact is known to be affected by the target tissue's oxygen status. Hypoxia is an important factor contributing to the development of radioresistance and therapy failure.[17] Carbon ion beams have high linear energy transfer irradiation, which is less dependent on the target tissue's oxygen status (eg, ischemic area of the heart). Therefore, a medium dose of carbon ion or proton beam radiation to the arrhythmogenic substrates of the LV may potentially eliminate abnormal slow conduction or abnormal focal source in the substrates, which would work as an alternative treatment method in patients with catheter ablation-resistant ventricular tachycardia/ventricular fibrillation. When using this treatment, the radiation area should be focused as narrowly as possible to avoid radiation of the surrounding normal tissues.

Limitations

First, this study was performed in healthy rabbits. The tissue characteristics of rabbits' hearts are not same as in humans, which would affect the electrophysiological and pathological changes. Second, we could perform only epicardial, and not endocardial, electrophysiological mapping in the LV because a rabbit's heart is too small to place the mapping catheter into the LV endocardium. Third, particle beam radiation in the present study was not performed in synchronization with the beating and respiratory motions of the heart because the heart rate was so rapid. However, the target radiation area was large enough to cover most of the LV, which minimized the risk of underdosed radiation in the target area. Fourth, significant LV dysfunction was not observed in the radiation rabbits. However, heart diseases (heart failure, cardiovascular events) have been reported to occur in patients many years after the completion of radiation therapy.[18] A long follow-up of LV function is needed after performing particle beam radiation in patients.

CONCLUSIONS

A medium dose of carbon ion and proton beam radiation resulted in significant electrophysiological and pathological changes in LV myocytes by 3 months after radiation. These effects were sustained at the 6-month follow-up. Radiation of the arrhythmogenic substrate would modify the electrical status and potentially induce the antiarrhythmic effect. When using this treatment, the radiation area should be focused as narrowly as possible to avoid radiation pneumonitis and pericardial effusion.

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Affiliations

From the Division of Cardiovascular Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan (M.T., T.H., H.S., T.A., M.T., T.N., J.S., A.Y., K.N., Y.S., K.Y., H.T.); Department of Radiology (T.O.) and Department of Radiation Physics (M.S.), Hyogo Ion Beam Medical Center, Tatsuno, Hyogo, Japan; and Section of Arrhythmia, Division of Cardiovascular Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan (K.F., K.K., K.H.).

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Disclosures

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