Minimal coronary artery damage by myocardial electroporation ablation

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Aims
Radiofrequency catheter ablation is a successful treatment for cardiac arrhythmias, but may lead to major complications such as permanent coronary damage. Irreversible electroporation (IRE) is a new non-thermal ablation modality, but its effect on coronary arteries is still unknown.

Methods and results
In a porcine model, epicardial IRE lesions were created at the base of the left ventricle in four hearts (group A) and directly on the left anterior descending artery (LAD) in five hearts (group B). After 3 weeks, coronary arteries inside IRE lesions and in apparently undamaged myocardium next to the lesions were (immuno-)histologically studied. Two untreated hearts served as controls. Coronary damage was defined as intimal hyperplasia. Left anterior descending artery angiograms were obtained before ablation, directly after ablation, and before termination in group B. In group A, 103 arterial branches were studied. Of these, 5 of 56 arterial branches inside lesions and 1 of 47 outside lesions showed intimal hyperplasia, but all had 50% area stenosis. Targeted LADs (group B) did not reveal intimal hyperplasia and angiograms showed no signs of stenosis. Expression of connective tissue growth factor was observed in the scar tissue, but not in the fibrotic tissue directly around the arteries, confirming that the arteries are indeed spared from tissue damage and remodelling.

Conclusion
Coronary arteries remain free of clinically relevant damage 3 weeks after epicardial IRE ablation, even amid very large myocardial lesions. This suggests that IRE ablation can be applied safely near or even on coronary arteries. With IRE ablation, arterial blood flow does not appear to affect lesion formation.

Keywords
Coronary artery • Catheter ablation • Lesion • CTGF • Electroporation

Introduction
Radiofrequency (RF) catheter ablation is a technique that was introduced in cardiac electrophysiology in 1987 and evolved from a highly experimental procedure to a standard treatment option for cardiac arrhythmias.¹

Radiofrequency induces heat damage to all tissue types near the ablation site. Ablation near coronary arteries therefore has serious risks.²,³ Radiofrequency heating may not only coagulate blood inside the vessel, but also causes intimal hyperplasia and shrinkage of the collagen fibres in the arterial wall. This may lead to vessel stenosis and subsequent infarction of the perfused territory.⁴ Conversely, tissue cooling by arterial flow may limit lesion formation and lead to unsuccessful ablations.⁵,⁶ Both thermal side effects are relevant not only for endocardial catheter ablation, but also for epicardial catheter ablation inside the pericardial space.⁷,⁸

Recently, circular irreversible electroporation (IRE) ablation has been proposed as a new catheter ablation modality.⁹ Irreversible electroporation uses direct current (DC) to create myocardial lesions. It is based on low-energy DC catheter ablation, a technology that avoids the cascade of potentially harmful side effects, such as the generation of vapour globe, sparking, explosion, and pressure waves associated with standard DC catheter ablation.¹⁰–¹³

Low-energy DC ablation, however, was abandoned shortly after its introduction when RF ablation became available.

Irreversible electroporation was tested and the first results demonstrated that it is capable of creating huge myocardial lesions within a few milliseconds, apparently without

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side effects. With such large lesions, the effect of the application on coronary arteries certainly has to be investigated before further development to a clinically applicable technology.

Tissue damage by IRE is the result of an electrical field-driven reorganization of the lipid structure of the cell membrane. This causes formation of permanent microlesions in the membrane that increase cellular permeability. This disrupts cellular homeostasis and initiates apoptosis of the cell. Other parts of the cell, such as DNA, collagen, or other proteins are not directly affected by IRE. The amount of damage caused by IRE depends on the presence of cell membranes, the applied electrical field, and the electrical resistivity of the target tissue. Therefore, some tissues may be more vulnerable for IRE than others. In oncological IRE application, it has been demonstrated that vital structures such as vessels remain relatively spared.

The aim of the present study was to analyse the effect of IRE on coronary arteries to pave the way for development of IRE catheter ablation in the pericardial space.

Methods

Animal experiments

All animal experiments were performed with approval from the Animal Experimental Ethical Committee of the University Medical Center Utrecht. In nine 60–75 kg pigs, median sternotomy was performed and the pericardium was opened. Its edges were lifted and sutured to surrounding instrumentation. A Starfish Heart Positioner (Medtronic Inc., Minneapolis, MN, USA) was used to raise the apex and expose the basal left ventricular (LV) area. Three custom devices (types A– C) were used for circular or linear IRE ablations on LV epicardium (Figure 1).

In a first series of four pigs, circular IRE was performed with device type A or B. The device was sutured around the LV base, the heart was lowered into the pericardial sac, and the pericardium was filled with heparinized blood. Energy was applied between the device and a remote indifferent electrode using a monophasic external defibrillator (Lifepak 9, Physio-Control, Inc., Redmond, WA, USA). The applied energy ranged between 50 and 360 J. This procedure was repeated at three other sites around the LV base, without overlap between lesions.

In a second series of five pigs, on the antero-septal part of the heart, circular IRE applications were purposely delivered directly on the left anterior descending artery (LAD) by positioning the ablation hoop over the LAD using devices A and B at an energy level between 50 and 200 J. In four of five animals, one or two additional single linear applications were delivered more distally on the LAD using device C at a lower setting of 30 J, because the suction prevented energy leakage to the surrounding blood pool.

After these ablations, the thorax was closed and the animal was allowed to recover. After 3 weeks the animals were terminated and the hearts were collected. Two hearts from untreated pigs served as controls.

In the second series of five animals, where ablations were performed directly on the LAD, coronary angiography was performed shortly before the ablation, within 60 min after ablation and before termination 3 weeks later. These angiograms were analysed for arterial stenosis.

Histological investigation

The hearts of the nine pigs treated with IRE and the two control hearts were fixated in formalin. From each heart, between one and nine consecutive 4 mm thick segments were dissected from each lesion. In the control hearts, an area similar to the treated hearts was used for histological analysis. The segments were embedded in paraffin, sectioned, and stained with haematoxylin–eosin and elastic-Van Gieson.

All histological sections were scanned with a ScanScope XT scanner (Aperio Technologies, Inc., CA, USA) and analysed using Imagescope (Aperio Technologies). Lesion depths were measured in the nine ablated hearts. Of all coronary arteries and branches found in sections of the first series of four hearts where ablation was performed at the base of the LV, the luminal area, the area encompassed by the internal elastic lamina (IEL area), and the area encompassed by the external elastic lamina (EEL area) were measured. The intimal area was calculated by subtracting the luminal area from the IEL area. All arteries with an EEL area > 0.15 mm² were considered clinically relevant and included in the study. Of the five hearts of the second series, where ablations had been performed directly on the LAD, only sections containing the LAD were analysed. Coronary damage was defined as intimal hyperplasia and the percentage stenosis due to the intimal hyperplasia was calculated as follows: (IEL area − luminal area)/IEL area × 100%.

In a subset of LV basal sections obtained from lesions and control hearts, connective tissue growth factor (CTGF) expression was determined using immunohistochemistry with a mouse anti-human CTGF monoclonal antibody (SC-14939; Santa Cruz Biotechnologies, Inc., CA, USA). Connective tissue growth factor is a pro-fibrotic growth factor that can be used as a biomarker for active tissue remodelling.

Statistical analysis

Difference in coronary artery size and lesion depth between affected and unaffected arteries was analysed with an unpaired t-test. The statistical significance was defined as a P value ≤ 0.05.

Results

All animals survived the procedure and the 3 weeks follow-up period. One animal suffered from an episode of fever, presumably due to pericarditis. No other complications were reported.
In the first series of five animals, 81 sections from 16 IRE lesions were analysed. The lesion depth was 6.5 ± 2.7 mm (range 1.7–13.5 mm). In 26 of 81 lesion sections, the lesion was transmural. The transmural lesions were mainly found in ablations with relative high-energy levels. As expected, arterial branches were predominantly located epicardially, sometimes very close to the application site. A total of 103 arterial branches with an EEL area >0.15 mm² were found. These arteries were subdivided into 56 arteries that were located within a lesion and 47 that were located outside a lesion. None of the arteries inside the lesion were surrounded by intact myocardial tissue. In 15 control sections of untreated hearts, 30 arteries with an EEL area >0.15 mm² were found. Major coronary branches (EEL area >1 mm²) were present inside lesions (n = 6), outside lesions (n = 12), and in control sections (n = 6) (Figure 2A and B).

Intimal hyperplasia was observed in 5 of 56 arteries inside lesions, in 1 of 47 arteries outside the lesions, and in 0 of 30 arteries from control sections (Figure 2E). The single affected artery outside a lesion had an EEL area of 0.16 mm². This artery was located 11 mm outside the lesion border and was surrounded by several small and large unaffected arteries. In all affected arteries, stenosis was <50%, with an average of 22 ± 15%. Lesions depth did not differ significantly between lesions with and without affected arteries (7.4 ± 3.3 vs. 7.2 ± 2.7 mm, respectively, P = 0.7). Affected arteries were significantly smaller than unaffected arteries inside the lesion (average EEL area of 0.35 ± 0.13 vs. 0.69 ± 0.85 mm², respectively, P < 0.001). None of the large coronaries (EEL area >1 mm²) was affected.

In the second series of four animals, 29 histological sections of five LAD lesions were analysed. All LADs were fully surrounded by the electroporation lesion. Lesions were 2.9 ± 1.2 mm deep (range 0.2–6.3 mm). In none of these LAD sections, intimal hyperplasia was found (Figure 2F). Left anterior descending artery coronary angiograms showed no signs of stenosis directly after ablation or before termination (Figure 3 and Table 1).

In sections stained for CTGF, the expression was observed in fibroblasts of scar tissue, confirming the existence of a remodelling process in the lesion. Strikingly, in the areas directly around coronary branches, CTGF expression was not detected (Figure 2G and H). The medial and intimal arterial layers of lesion and control sections showed a diffuse expression of CTGF.

Discussion

In this study, we investigated the effect of various types of epicardial IRE ablation on coronary arteries. Electroporation energy was delivered epicardially with blood around the electrode–tissue contact site to simulate the situation during endocardial catheter ablation.

The data suggest that coronary arteries do not develop significant stenosis within 3 weeks after epicardial IRE, even not amid large LV lesions and despite their epicardial location near the ablation electrode(s).

In RF and cryo-energy ablation, coronary damage is a rare, but well-recognized complication in epicardial ablation procedures. Acute coronary damage as well as damage several weeks after an RF ablation procedure requiring interventions such as coronary stenting have been described. Several mechanisms explain the occurrence of coronary damage after RF. First, RF heat induces mechanical damage to all tissue types near the ablation site. If ablation is applied in the proximity of a coronary artery, the artery will also be damaged. Secondly, animal models demonstrate that heat from RF can induce clot formation in the coronary vessels which may lead to an acute coronary artery occlusion. Clots are caused by protein aggregation, which is initiated by blood temperatures >50°C. Thirdly, heat of RF denatures collagen in coronary artery walls, which accounts for induced vessel constriction and subsequent coronary damage. During RF ablation, coronary angiography is routinely performed to exclude the presence of major arteries near the target site. During endocardial catheter ablation, the risk of ablation in close proximity to a coronary artery is smaller, but even then coronary artery damage has been reported. Irreversible electroporation is a new ablation modality that creates large myocardial lesions within a few milliseconds. Formation of large lesions involves a significant risk of coronary artery presence in the ablation area. Our observations suggest that IRE may be a relatively safe ablation modality for epicardial ablation near coronary arteries and that coronary angiography before epicardial ablation may no longer be required when using this ablation technique.

Connective tissue growth factor [also known as CCN2 (Cyr61/CTGF/NOV)] is a profibrotic growth factor that induces accumulation of extracellular matrix by inducing collagen production and inhibiting matrix breakdown. It is a protein that is involved in numerous physiological processes in several organs. In the heart, CTGF is expressed in remodelling processes involving myocardial fibrosis, such as heart failure, myocardial infarction, and myocardial damage due to chronic hypertension. In vivo, there is a strong association between cardiac fibrosis and CTGF expression. In the current study we therefore considered CTGF a biomarker for active remodelling of scar tissue and used it to compare remodelling in different parts of the IRE lesion. Indeed, CTGF expression was found in the fibroblasts of the lesions caused by IRE. In the areas that directly surround coronary arteries (the adventitia), however, no CTGF expression was found. This suggests the absence of remodelling and scar tissue formation in these parts of the lesion. A diffuse expression of CTGF was observed in the medial and intimal layers of the arteries inside lesions and in controls. From previous studies it is known that a basal CTGF expression without an underlying pathological process is present in these artery layers. This was confirmed by the fact that we also observed this expression pattern in control arteries. The observed CTGF expression pattern suggests that not only the arteries, but also connective tissue around coronary arteries remains unaffected by IRE.

Lesion formation in RF and cryoablation is a thermal process and logically affected by arterial and venous blood flow cooling, the so-called cold/heat sink effect. Blood flow protects the artery from thermal damage, but also cools or warms myocardial tissue in its direct vicinity. This may lead to incomplete lesion formation, gaps in ablation lines, and arrhythmia recurrences necessitating multiple ablation sessions. Theoretically, lesion formation in IRE is not temperature related and indeed inside lesions we did not find intact myocardial tissue around blood vessels. Data
of the present study therefore suggest that with IRE ablation, coronary arteries are not or only minimally affected by the application and conversely that myocardial lesion formation does not appear to be affected by the presence of major arteries. These are two reasons why IRE may be a much better ablation method than RF for targeting an arrhythmogenic substrate near coronary arteries.

**Figure 2** Histological analysis of myocardial tissue after electroporation ablation. (A) Haematoxylin–eosin stain of an epicardial lesion. The area in the middle is the electroporation lesion, the pink area on both sides is undamaged myocardium. Bar = 1.25 mm. (B) Elastic-Van Gieson stain of the same lesion as in (A). Inside the lesion are several small arterial branches and two large undamaged coronary branches present. Bar = 1.0 mm. (C and D) Elastic-Van Gieson stain of transmural lesions. * Indicates the epicardial side of the heart and # the endocardial side of the heart. C, Bar = 2.8 mm; D, Bar = 2.3 mm. (E) Elastic-Van Gieson stain of two examples of large coronary arteries in an electroporation lesion; one with intimal hyperplasia on the left and one without intimal hyperplasia on the right. Bar = 0.25 mm. (F) Elastic-Van Gieson stain of an undamaged left anterior descending artery surrounded by electroporation lesion. The pink area is the ablation lesion, the brown area at the bottom of the figure is intact myocardium. Bar = 0.7 mm. (G) Connective tissue growth factor expression in fibroblasts. Bar = 0.035 mm. (H) Connective tissue growth factor expression around coronary artery branches. * shows connective tissue growth factor expression in electroporation lesion, † indicates the area around vessels where CTGF is absent. Bar = 0.070 mm.
There are two possible explanations for the preservation of coronary arteries. Connective tissue around coronary arteries has an extensive extracellular network and exhibits a relative reduction in large cell membranes, which may make them relatively invulnerable to IRE. An alternative explanation may be that connective tissue around coronary arteries has a relatively high electrical resistivity. This may protect the medial and intimal arterial layer against high electrical fields.

Limitations

Analysis of coronary arteries was limited to arteries with an EEL area ≥ 0.15 mm². This limit was arbitrarily chosen before the analysis because of the abundance of very small coronary artery branches within the myocardium. Another limit may have yielded different results, especially since the affected arteries found in the current study were relatively small. However, stenosis of tiny arteries and arterioles is clinically less relevant and might be secondary to the lesion formation as part of a remodelling process.

Lesions that resulted from IRE applications directly on the LAD were relatively small. In part this may be due to the presence of epicardial fat with a higher electrical resistivity resulting in reduced local current density and thus limited lesion extension. This latter aspect requires further study. The LADs were completely surrounded by electroporation lesion and other transmural lesions show large intact coronary artery branches near the ablation electrode. Nevertheless, higher energy applications that cause transmural lesion directly on the LAD may be required to strengthen the evidence that IRE application directly on major arteries is safe.

The follow-up period was limited to 3 weeks. The long-term effects of IRE on coronary arteries and the role of remodelling therefore remain unknown. However, the absence of CTGF expression in arterial layers and surrounding connective tissue suggests that these arteries are not affected by IRE ablation. Longer follow-up periods may be required to analyse the long-term effect of IRE on coronary arteries, but the absence of CTGF expression suggests that a late response is unlikely.

Coronary damage was defined as intimal hyperplasia because this is the clinically most appropriate marker of coronary damage. Others have shown that the vascular smooth muscle cells in the medial layer of the artery may be affected by IRE. Although we did not systematically score medial thickness and the number of vascular smooth muscle cells in the medial layer, we did not observe evident pathological changes in the media. The data of our study therefore suggest that the medial layer is not affected at the settings used in the present protocol.

Different custom devices and different energy levels were applied to create myocardial lesions. In the current study, we filled the pericardial sac with blood to simulate the situation during endocardial catheter ablation where blood also surrounds the electrode–tissue contact sites. The relatively high conductivity of blood shunts most of the ablation current. With catheter ablations in the pericardial space in the absence of electrically shunting blood, a much lower energy level will be required to create similar lesions. However, the range of lesion sizes investigated in the present study is sufficiently broad for pericardial ablation and data of the present study are therefore believed to be relevant for such application.

Conclusions

Coronary arteries remain free of significant damage amid huge transmural myocardial lesions, 3 weeks after epicardial IRE ablation very near to these arteries. This suggests that IRE ablation has a low risk of coronary damage and that epicardial IRE ablation
Near or even on large coronary arteries is relatively safe. With IRE, myocardial lesion extension does not appear to be affected by the presence of arterial blood flow. This ablation modality may therefore be an appropriate solution for epicardial target sites near coronary arteries.

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Conflict of interest:

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