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Aging-related mitochondrial dysfunction facilitates the occurrence of serious arrhythmia after myocardial infarction

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ABSTRACT

Background: During aging a mosaic of normal cells and cells with mitochondrial deficiency develops in various tissues including the heart. Whether this contributes to higher susceptibility for arrhythmia following myocardial infarction (MI) is unknown.

Methods and Results: Myocardial cryoinfarction was performed in 12-month-old transgenic mice with accelerated accumulation of deletions in mitochondrial DNA. Occurrence and pathogenesis of arrhythmia was investigated after two weeks. Holter-ECG recordings revealed higher rates of premature ventricular complexes (incidence > 10/24 h: 100% vs. 20%; $p = 0.048$) and more severe spontaneous arrhythmia during stress test in mutant mice with MI as compared to control mice with MI. Mice with mitochondrial dysfunction exhibited longer spontaneous AV-blocks (467 ± 26 ms vs. 377 ± 24 ms; $p = 0.013$), an increased probability for induction of ventricular tachycardia during in vivo electrophysiological investigation (22% vs. 9%; $p = 0.044$), and a reduced conduction velocity in the infarct borderzone (38.5 ± 0.5 cm/s vs. 55.3 ± 0.9 cm/s; $p = 0.001$). Furthermore, mutant mice exhibited a significant reduction of the phospho-Cx43/Cx43 ratio in right (0.59 ± 0.04 vs. 0.85 ± 0.01; $p = 0.027$) and left ventricular myocardium (0.72 ± 0.01 vs. 0.86 ± 0.02; $p = 0.023$).

Conclusions: Aging-related cardiac mosaic respiratory chain dysfunction facilitates the occurrence of spontaneous and inducible cardiac arrhythmia after myocardial infarction and is associated with slowing of electrical impulse propagation in the infarct borderzone.

1. Introduction

The process of aging is associated with a significant increase in various forms of cardiac arrhythmia, namely atrio-ventricular (AV) blocks, right and left bundle branch blocks, supraventricular and ventricular premature beats and atrial fibrillation [1–3]. Although the underlying mechanisms are not clear to date, this has in part been linked to an age-related increase in interstitial fibrosis, especially in the right ventricle, resulting in decreased cell coupling and reduced conduction velocities, therefore facilitating re-entrant arrhythmia [4,5]. Stein and colleagues could demonstrate that medical inhibition of the renin-angiotensin-aldosterone system reverses fibrosis and thereby reduces inducibility of ventricular arrhythmia in the aged mouse model [6].

Aging is also associated with mitochondrial dysfunction and it is well known that in aged human hearts, like in other tissues, individual cardiomyocytes with dysfunctional mitochondria

Abbreviations: ARP, atrial refractory period; AV, atrio-ventricular; AVNRP, AV-nodal refractory period; CL, cycle length; MI, myocardial infarction; PVC, premature ventricular complexes; SNRP, sinus node recovery period; VT, ventricular tachycardia; VRP, ventricular refractory period; WBP, Wenckebach periodicity.

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accumulate [7,8]. Mitochondrial dysfunction has been associated with arrhythmia in patients undergoing cardiac surgery [9]. We could recently demonstrate in a transgenic mouse model, that mitochondrial dysfunction of only few cardiomyocytes promote arrhythmia during aging [10]. To mimic the aging related mosaic respiratory chain deficiency in mouse, a dominant-negative mutant of the mitochondrial replicative helicase Twinkle was expressed in the myocardium (K320E-Twinkle^{Myo} mice), therefore leading to an accelerated accumulation of mtDNA deletions in this tissue. As a consequence, a cellular mosaic consisting of many healthy and only a few cells with mitochondrial dysfunction (COX^{neg} cells, assessed by staining for cytochrome C oxidase activity) developed. At the age of 18 months, K320E-Twinkle^{Myo} mice showed an increase in premature ventricular contractions (PVCs) and AV-blocks during a physical exercise test as compared to control mice, while no significant differences could be observed at the age of 12 months.

The prevalence of coronary artery disease and myocardial infarction (MI) also increases with age [11]. However, there is insufficient data concerning the interaction between normal aging-related myocardial changes and ischemic heart disease. Therefore, we induced MI in 12 month-old K320E-Twinkle^{Myo} mice, which at baseline show no arrhythmic abnormalities, and performed Holter-ECGs and invasive electrophysiological investigations to test for spontaneous and inducible arrhythmia.

2. Material and methods

2.1. Animals and procedures

Male and female, 12 month-old mice (genetic background: C57BL/6J) were used for this study. The generation of K320E-Twinkle^{Myo} mice has been described previously [10]. Littermates with the genotype R26-K320E-Twinkle$^{loxP/+}$ were used as controls. The animals were maintained under standardized housing conditions (22 °C with a 12 h light/dark cycle, drinking water ad libitum).

The animal experiments were carried out according to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. The investigations were approved by the National Office for Nature, Environment and Consumer Protection in Recklinghausen, Nordrhein-Westfalen (Permit Number: 84–02.04.2013.A165).

2.2. Cryoinfarction

Myocardial cryoinfarction was performed under general anesthesia as previously described [12–14]. The cryoinfarction technique was deliberately chosen in order to obtain comparable results with similar infarct sizes and circumscribed borderzones [13]. In brief, mice were anesthetized, analgized, intubated and mechanically ventilated (Harvard Apparatus Rodent Ventilator Mod. 681, Harvard Apparatus Inc.). Following left lateral thoracotomy the pericardium was incised, the heart exposed and a transmural cryolesion generated on the anterior free left ventricular wall (copper probe, 3 mm diameter, −200 °C, procedure repeated 3 times). After wound closure the remaining pneumothorax was manually drained.

2.3. Electrophysiological investigation (EPI)

Electrophysiological investigations were performed under analgesia and inhalation anesthesia two weeks after cryoinfarction as reported previously [15–18]. In brief, a 2 French octapolar mouse electrophysiological catheter (Cib'er Mouse, NuMed Inc., New York, USA) was inserted via the jugular vein in the right heart chambers with the proximal electrodes on atrial and the distal electrodes on ventricular level. Additionally, a 6-lead surface ECG was recorded for calculation of standard ECG parameters.

Table 1
Baseline parameters. Baseline characteristics at time point of electrophysiological investigation. All values are mean ± s.e.m.

	MI control n = 9	MI K320E-Twinkle^{Myo} n = 11	p-value
male [%]	67	73	1.00
body weight [g]	32.6 ± 0.7	30.8 ± 0.8	0.14
heart weight [mg]	240.1 ± 19.0	226.2 ± 12.3	0.53
heart/body weight [mg/g]	7.4 ± 0.7	7.3 ± 0.3	0.93

Table 2
Holter-ECG recordings. ECG parameters from Holter-ECGs during 24 h of rest or 10 min of swimming exercise (stress). QTc: rate corrected QT interval; PVC: premature ventricular contraction. All values are mean ± s.e.m.*p < 0.05 for MI *versus* no MI; #p < 0.05 for rest *versus* stress.

	control n = 5	K320E-Twinkle^{Myo} n = 5	MI control n = 5	MI K320E-Twinkle^{Myo} n = 5
Rest				
heart rate [bpm]	622.8 ± 17.4	590.1 ± 14.3	626.2 ± 13.2$^{\#}$	613.4 ± 15.4$^{\#}$
P-duration [ms]	14.5 ± 1.5	15.5 ± 0.5	13.4 ± 1.0	13.4 ± 0.9
PR-interval [ms]	39.4 ± 1.1*	39.2 ± 0.6	36.0 ± 0.8*	39.0 ± 1.4
QRS-interval [ms]	11.4 ± 0.3*	10.6 ± 0.3	10.2 ± 0.4*	10.4 ± 0.2
QT-interval [ms]	41.4 ± 1.1* #	45.7 ± 1.7*	51.6 ± 2.1*	53.4 ± 1.6*
QTc-interval [ms]	42.3 ± 1.1* #	46.0 ± 1.6* #	53.4 ± 1.3*	53.2 ± 1.7* #
PVC/h	0.06 ± 0.02	0.16 ± 0.05*	2.54 ± 2.39	21.75 ± 19.57*
AV-block/h	0.00 ± 0.00	0.02 ± 0.02*	0.1 ± 0.1	0.2 ± 0.1* #
Stress				
heart rate [bpm]	672.3 ± 12.1	646.5 ± 40.0	702.0 ± 5.6$^{\#}$	693.8 ± 13.5$^{\#}$
P-duration [ms]	16.3 ± 2.3	13.0 ± 1.5	14.8 ± 1.3	12.0 ± 0.8
PR-interval [ms]	38.6 ± 1.2	38.1 ± 1.9	35.9 ± 1.4	38.0 ± 1.9
QRS-interval [ms]	11.1 ± 0.3	11.4 ± 0.8	10.8 ± 0.6	10.8 ± 0.6
QT-interval [ms]	44.0 ± 1.4$^{\#}$	48.4 ± 1.2	47.3 ± 1.0	45.4 ± 1.9
QTc-interval [ms]	46.5 ± 1.7* #	50.1 ± 1.3$^{\#}$	51.8 ± 0.9*	49.0 ± 2.0$^{\#}$
PVC/h	26.4 ± 5.2	28.8 ± 14.0	8.00 ± 8.00	19.20 ± 6.95
AV-block/h	8.4 ± 3.1	15.6 ± 5.9	18.0 ± 18.0	24.0 ± 3.3$^{\#}$

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