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Intra-myocardial growth hormone administration ameliorates arrhythmogenesis during ischemia–reperfusion in rats

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Abstract Growth hormone, currently under evaluation for the prevention of left ventricular remodeling post-myocardial infarction, displays antiarrhythmic properties in the acute setting. However, it is uncertain whether these actions are retained after ischemia/reperfusion. Using implanted telemetry transmitters, we examined the effects of prolonged, intra-myocardial growth hormone administration in conscious rats. During a 24-h observation period, ventricular tachyarrhythmias and sympathetic activation were attenuated in treated rats, whereas infarct-size was unchanged. These findings call for further study on the antiarrhythmic effects of growth hormone and on the underlying mechanisms.
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Introduction

The growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis has been implicated in the pathophysiology of acute myocardial infarction (MI) and subsequent left ventricular (LV) remodeling. Based on this rationale, attenuated LV dilatation and improved systolic function have been reported after systemic GH-administration in animal models [1]. As the benefits of GH in this setting appear secondary to its favorable actions on the infarct and peri-infarct zones, local administration has been advocated by several groups; further to this concept, we recently demonstrated decreased wall-stress after prolonged local GH-delivery via an implanted biomaterial-scaffold in rats, leading to improved remodeling indices [2].

In addition to the long-term effects, potent antiarrhythmic actions were evident in rats pretreated with GH after non-reperfused acute MI [3,4], a setting characterized by high incidence of ventricular tachyarrhythmias (VTs). Although this finding introduces a pathophysiologic proof-of-concept, the effects of local GH-delivery on arrhythmogenesis during acute MI treated with reperfusion remain uncertain; such information is important in the evaluation of the potential clinical benefit of GH, in the present era of widely employed primary percutaneous interventions.

Using the in vivo ischemia/reperfusion rat-model, we aimed to examine the effects of GH, delivered locally via an alginate-based scaffold, on VTs during a 24-h observation period. Potential cytoprotective treatment-effects were assessed by measuring infarct size and voluntary activity, the latter used as a surrogate-marker of acute heart failure. Lastly, sympathetic activation, an important arrhythmogenic mechanism, was examined by analyzing indices of heart rate variability (HRV).

Material and methods

Animal population and ethics

The experiments were conducted on 36 Wistar rats (18–20 weeks of age, weighing 280-380 g), a useful animal-model in the study of ischemia-induced VTs. The animals were housed in Plexiglas-cages in groups of two and received humane care; laboratory conditions were kept optimal (in terms of temperature, humidity and light:dark cycles), and access to water and rodent pellet-diet was provided at all times. The study-protocol conformed to European legislation (2010/63/

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EU) and was approved by the responsible regulatory state-authorities.

Fabrication of scaffold

The scaffold was based on alginate-hydrogel, prepared by dissolving alginic-acid sodium-salt (FMC, Biopolymers, Oslo, Norway) in normal saline, under magnetic stirring and ultrasonic agitation. A second solution was prepared by dissolving D-(+)-gluconic acid δ-lactone (Sigma-Aldrich, St. Louis, MO, USA) and dehydrated CaCl2 (Fluka Analytical, Sigma) in normal saline; it was added drop-wise into the first solution under continuous stirring, allowing controlled cross-linking. Finally, dissolved GH (Sigma) was added drop-wise, and the obtained hydrogel was stored in 0.2-ml aliquots, each containing 2.5 μg of GH.

Implantation of telemetry transmitters

After tracheal intubation and mechanical ventilation (with a rodent apparatus model 7025, Ugo Basile, Comerio, Italy), the rats were anesthetized with a mixture of oxygen and 2.5% sevoflurane. Continuous electrocardiographic (ECG) monitoring was performed with the use of miniature telemetry transmitters (TCA-F40, Data Sciences International, *DSI*, Arden Hills, MN, USA), which permit long-term recording in conscious, unrestricted animals, thus circumventing the confounding effects of anesthesia. The transmitters were implanted in the abdominal cavity, with the two leads secured under the right axilla and at the left hind-limb area, respectively; the animals were placed on a receiver (RCA-1020, *DSI*), continuously capturing the ECG-signal that was saved for analysis.

Ischemia–reperfusion and scaffold implantation

The left coronary artery was encircled, midway between its origin and the apex, with a 6-0 suture threaded through a snare. Tightening the snare induced MI, validated by ST-segment elevation in a 6-lead ECG (QRS-Card digital PC-ECG, Pulse Biomedical Inc., *PBI*, Norristown PA, USA) after amplification by software (Cardiology Suite ver. 4.05, *PBI*); after 30 min of ischemia, reperfusion was attained by releasing the snare. After 10 min of reperfusion, alginate-hydrogel (0.2 ml, with or without GH) was administered by six intra-myocardial injections around the antero-lateral LV wall. The following groups were randomly formed: (A) control-group (n = 12), which received 0.2 ml of normal saline; (B) alginate-injections (scaffold-group, n = 12); (C) injections of alginate containing GH (GH-group, n = 12). The incision was then rapidly closed, pneumothorax was evacuated, and the animals regained consciousness within ~3 min after anesthesia discontinuation.

Infarct size

The risk- and infarcted-zones were measured at the end of the 24-h observation period; specifically, the heart was mounted on a perfusion-apparatus, the coronary ligature was tightened, and a saline-solution of green-fluorescent microspheres (Duke Scientific Corp., Palo Alto, CA, USA) was infused over 5 min. After freezing at −20 °C for 24 h, the samples were sliced into 2-mm sections, which were incubated in 1% triphenyltetrazolium chloride for 20 min; finally, they were immersed in 10% formaldehyde solution for further 24 h. The infarcted and ischemic zones were identified under ultraviolet light (λ = 366 nm) and measured with ImageJ (NIH, Bethesda, MD, USA); infarct size was expressed as the ratio of the volumes of the two zones.

Mortality, arrhythmia-analysis and activity

The survival duration was accurately documented from ECG-recordings. VTs (i.e., monomorphic or polymorphic ventricular tachycardia and ventricular fibrillation) were identified, and the duration of each episode was measured; the total number and duration of VT-episodes are reported for the entire 24-h observation period. Lastly, we recorded the number of strength-variations in the telemetry-signal in relation to animal location, reflecting voluntary motor-activity, which correlates with the incidence and severity of heart failure.

Indices of sympathetic activation

Heart rate (HR) was calculated from consecutive RR-intervals, after exclusion of non-sinus beats, and the average value is reported. Sympathetic activation was further evaluated by measuring HRV-indices, aided by the Kubios-software (version 2.1, Biosignal Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern Finland, Kuopio, Finland), i.e., (a) the ratio of low- (LF, 0.195–0.605 Hz) to high-frequency (HF, 0.605–2.5 Hz) bands, which depicts the sympatho-vagal balance, and displays good correlation with plasma catecholamines; (b) the dispersion of Poincaré plots after nonlinear analysis, reflecting mainly vagal activity.

Statistical analysis

Values are reported as mean ± standard error of the mean. Kaplan–Meier survival curves were assessed with χ^2 for heterogeneity, followed by Peto & Peto's Wilcoxon test. Differences in normally distributed continuous variables (as per Kolmogorov–Smirnov test) were compared with the analysis of variance, followed by Duncan's multi-stage test. After Box–Cox transformation to normality, these tests were also used for the comparison of the number and duration of VT-episodes, exhibiting highly skewed distributions. Statistical significance was defined at an alpha-level of 0.05.

Results

Implantation procedure and survival

Implantation was uneventful in all rats, with only minor bleeding occasionally encountered at the puncture site. No heterogeneity was present between the three groups in the survival curves during the 24-h observation period, and no significant difference (p = 0.13) was seen between GH-treated rats and controls.

Infarct size and activity

Infarct size (as percent of the ischemic-zone) was comparable in the three groups (F = 0.2, p = 0.7); specifically, it was 34 ±

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