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RESEARCH PAPER

Attenuation of post-infarction remodeling in rats by sustained myocardial growth hormone administration

Evangelos P. Daskalopoulos^{1,2*}, Agapi D. Vilaeti^{2*}, Eleonora Barka^{2,3*}, Polixeni Mantzouratou⁴, Dimitrios Kouroupis⁵, Marianthi Kontonika², Christos Tourmousoglou², Apostolos Papalois^{2,6}, Constantinos Pantos⁴, W. Matthijs Blankestijn¹, Simeon Agathopoulos³, and Theofilos M. Kolettis²

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Abstract

Prevention of left ventricular remodeling is an important therapeutic target post-myocardial infarction. Experimentally, treatment with growth hormone (GH) is beneficial, but sustained local administration has not been thoroughly investigated. We studied 58 rats (322 ± 4 g). GH was administered via a biomaterial-scaffold, following *in vitro* and *in vivo* evaluation of degradation and drug-release curves. Treatment consisted of intra-myocardial injection of saline or alginate-hydrogel, with or without GH, 10 min after permanent coronary artery ligation. Echocardiographic and histologic remodeling-indices were examined 3 weeks post-ligation, followed by immunohistochemical evaluation of angiogenesis, collagen, macrophages and myofibroblasts. GH-release completed at 3 days and alginate-degradation at ~ 7 days. Alginate + GH consistently improved left ventricular end-diastolic and end-systolic diameters, ventricular sphericity, wall tension index and infarct-thickness. Microvascular-density and myofibroblast-count in the infarct and peri-infarct areas were higher after alginate + GH. Macrophage-count and collagen-content did not differ between groups. Early, sustained GH-administration enhances angiogenesis and myofibroblast-activation and ameliorates post-infarction remodeling.

Keywords

Alginate, angiogenesis, growth hormone, myofibroblasts, myocardial infarction, ventricular remodeling

History

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Introduction

Myocardial infarction (MI) is a common clinical entity, associated with substantial morbidity and mortality. Despite reperfusion that salvages contractile tissue, extensive myocardial necrosis results in left ventricular (LV) remodeling and dysfunction, with ultimate transition to chronic heart failure (Sutton & Sharpe, 2000). Triggered by elevated wall-stress, this process initiates shortly after acute coronary occlusion, leading to infarct thinning and expansion, with subsequent dilatation of the non-infarcted zones (Agathopoulos & Kolettis, 2014). As these changes occur mostly during the early post-MI period (Ertl & Frantz, 2005), interventions targeting infarct expansion may prevent subsequent global alterations in LV architecture and function (Mitsi et al., 2005).

Several lines of evidence implicate the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis as an important

regulator of post-MI remodeling (Friberg et al., 2000). Accordingly, experimental studies have shown attenuated LV dilatation, improved LV function and lower mortality after systemic GH-administration post-MI (Cittadini et al., 1997, 2003; Jin et al., 2002; Omerovic et al., 2000). Local delivery in the infarct-zone has been advocated by our group (Hatzistergos et al., 2008) and others (Jayasankar et al., 2004), because it maximizes efficacy and avoids systemic effects; for example, acute intra-coronary administration of GH (Mitsi et al., 2006) or IGF-1 (O'Sullivan et al., 2011) in the porcine MI-model produced long-term beneficial effects on LV architecture and function. However, it is unknown whether this action can be potentiated, if treatment is sustained during the critical early post-MI period.

During the past decade, biomaterials have been extensively evaluated in cardiac-repair strategies, based on their chemical and physical properties that permit their use as tissue-engineering constructs; moreover, biomaterials can be viewed as ideal candidates for controlled and sustained local delivery of various agents, thereby prolonging their therapeutic actions (Kolettis et al., 2011). Based on this concept, we hypothesized that, local myocardial GH-administration via a biodegradable

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scaffold may enhance the effects of each individual approach and may prevent adverse LV alterations, if initiated immediately post-MI. We opted to use alginate, a naturally occurring, water-soluble polysaccharide co-polymer with gel-forming features, that meets most requirements for cardiac tissue-engineering (Augst et al., 2006). Alginate can be fabricated in liquid form *in vitro*, enabling the addition of proteins, but after intra-myocardial injection *in vivo*, it cross-links with calcium ions and undergoes transition into a viscous scaffold (Rowley et al., 1999). Due to these properties, alginate exerts favorable effects on LV remodeling in the rat (Landa et al., 2008) and porcine (Mukherjee et al., 2008) MI-models. Furthermore, such treatment appears safe, in terms of acute-phase arrhythmogenesis and short-term mortality (Kontonika et al., 2014).

In this study, we examined the value of sustained, local GH-release via an alginate-hydrogel on post-MI LV remodeling in the rat-model. Enhanced myocardial contractility and decreased apoptosis mediate the benefits of GH-treatment in the post-MI setting (Cittadini et al., 1997; Cittadini et al., 2003), but additional mechanisms are likely (Khan et al., 2001). Thus, based on previous evidence (Cook et al., 1988; Lee et al., 2010; Struman et al., 1999), we aimed to shed further light into myofibroblast-activation and angiogenesis.

Methods

Fabrication of biomaterials

Alginate-hydrogel was prepared by dissolving alginic-acid sodium-salt (180947, Sigma Aldrich, St. Louis, MO) in normal-saline, using magnetic stirring and ultrasonic agitation (solution A). A second solution (solution B) was prepared by dissolving D-(+)-gluconic acid δ -lactone ([GDL], G2164, Sigma Aldrich) and dehydrated CaCl_2 (21074, Fluka Analytical, Sigma Aldrich) in normal-saline. An equal volume (200 μl) of solution B was added drop-wise into solution A, under continuous stirring with a micropipette, allowing chelation of calcium ions with α -L-guluronic acid residues; this method controls cross-linking and results in a well-developed network (Augst et al., 2006). For alginate + GH, GH (S8648, Sigma Aldrich) was dissolved in normal-saline and was added dropwise into solution A, under constant magnetic stirring for 30 min, followed by the addition of solution B. The final alginate-hydrogel (concentrations: alginic acid 1% per weight; CaCl_2 4.2 mM; GDL 8.4 mM) was stored in 0.2 ml-aliquots; based on our previous work (Elaiopoulos et al., 2007; Hatzistergos et al., 2008), each alginate + GH aliquot contained 5 μg of GH.

In vitro degradation of alginate

In vitro degradation was determined on 5 ml alginate-hydrogel, laid gently on the bottom of a beaker. After adding 4 ml double-distilled water over its layer, the beaker was placed in an incubator at 37 °C; 3 ml of the supernatant liquid was sampled at 1, 3, 6, 12 and 24 h, and daily thereafter for a total of 8 days. The spectra were recorded with a UV-visible photometer (UV-Vis mini 1240, Spectrophotometer,

Shimadzu, Kyoto, Japan), and the absorption bands between 240 and 300 nm, characteristic of the alginate components (Nagasawa et al., 2000), were recorded. The concentration of alginate in the samples was calculated after fitting a calibration-curve from maximal absorption values.

GH-release rate measurements

GH-release from the alginate-hydrogel was examined with fluorescence spectroscopy (RF-5301 PC, Spectrophotometer, Shimadzu), which enables precise measurements at nano-scale. The specific assay lies on the intrinsic fluorescence probes of tyrosine and tryptophan residues in GH, based on their sensitivity to electronic environment. We recorded spectra at emission wavelength of 329 nm and excitation wavelength of 280 nm, with the calibration-curve fitted using distilled water and aqueous solutions of GH (at various concentrations). Subsequently, alginate-hydrogel samples with or without GH were examined at 1, 3, 6, 12 and 24 h, and daily thereafter for a total of 4 days.

Experimental animal-population and ethics

The *in vivo* part of the study was conducted on 58 Wistar rats (all male, 16–18 weeks of age, weighing 322 ± 4 g). Of these, 52 rats were subjected to MI, whereas six rats were sham-operated. The animals received humane care, according to the guiding principles of the Declaration of Helsinki (regarding ethical conduct of animal-research) and to European legislation (European Union directive for the protection of animals used for scientific purposes 2010/63/EU). They were housed in plexiglas-cages in groups of two, under optimal laboratory conditions regarding temperature (20–22 °C), humidity (~70%) and light:dark cycles (12:12); water and standard rodent pellet-diet were provided *ad libitum*. The study protocol was approved by the institutional review board and by the responsible regulatory state authorities.

In vivo degradation of alginate

Biotin was incorporated in alginate by carbodiimide-chemistry; specifically, biotin-hydrazide was added to the alginate-solution in 0.1 M MES buffer, at pH 6.0, under stirring for 60 min at room temperature. The resultant polymer was dialyzed against double-deionized water using a 10,000-MWCO-membrane; the water was changed twice daily for 3 days, followed by dropwise addition of 2.9 mM CaCl_2 . The biotin-alginate scaffold was characterized *in vitro* by FTIR spectroscopy.

The scaffold was implanted 10 min after MI-induction (see below) in a separate group of 10 rats (302 ± 6 g), which were compared with intra-myocardial injections of normal-saline ($n = 6$, 310 ± 5 g). The animals were sacrificed (as described below), three ($n = 3$ alginate, $n = 2$ saline), seven ($n = 4$ alginate, $n = 2$ saline) and 15 ($n = 3$ alginate, $n = 2$ saline) days after implantation. Biotin-labeled alginate was examined in 5 μm -sections, immunostained with streptavidin-HRP; the sections were photographed, and the areas of avidin-biotin positive staining were measured with ImageJ software (NIH, Bethesda, MD).

Animal groups

The treatment-effects on LV remodeling were examined in 42 rats (330 ± 5 g), randomly forming the following groups: (A) sham-operated animals, in which no MI was induced and no treatment was administered ($n = 6$), (B) control (MI-induction, followed by normal-saline intra-myocardial injections, $n = 12$), (C) alginate-gel intra-myocardial injections post-MI (alginate, $n = 12$); (D) intra-myocardial injections of alginate-gel containing GH post-MI (alginate + GH, $n = 12$).

In vivo experimental protocol

The animal body-weight was recorded at the beginning and at the end of the *in vivo* experiments. Treatment was administered 10 min after MI-induction; following echocardiographic assessment of LV remodeling three weeks post-MI, the rats were sacrificed and the hearts were excised for histological evaluation.

MI-induction

The animals were intubated and mechanically ventilated using a rodent-apparatus (model 7025, Ugo Basile, Comerio, VA, Italy); anesthesia was maintained with a mixture of oxygen and 2.5% sevoflurane. Through a left thoracotomy, the left coronary artery was encircled and ligated 4 mm from its origin with a 6-0 suture, extending from the pulmonary cone to the left atrial appendage, as previously (Kolettis et al., 2007); following these anatomical landmarks ensures comparable infarct size (Baltogiannis et al., 2005). The generation of MI was confirmed by the inspection of a pale, akinetic area and by ST elevation in a six-lead ECG (QRS-Card PC-ECG, Pulse Biomedical Inc., PBI, Norristown, PA), after amplification (Cardiology Suite version 4.05, PBI). In sham-operated animals, the left coronary artery was encircled, but not ligated.

Implantation procedure

The heart was exteriorized and slight traction was applied via a 6-0 suture in the apex, as before (Vilaeti et al., 2013). A total of 0.2 ml of normal-saline or alginate-hydrogel (with or without GH) was administered by six intra-myocardial injections in the antero-lateral LV wall, as in earlier experiments (Hatzistergos et al., 2008). The incision was closed in three layers and pneumothorax was evacuated; a single intra-peritoneal injection of an opioid-analgesic (buprenorphine, 0.05 mg/kg) was given, followed by antibiotic prophylaxis (cephazolin, 15 mg/kg daily), administered intra-muscularly for 5 days post-operatively.

Echocardiography

Echocardiography was performed three weeks post-MI, using previously described methodology (Pantos et al., 2008); briefly, under sedation with intra-peritoneal ketamine (100 mg/kg), short- and long-axis images were acquired using a digital ultrasound-system (Vivid 7, GE Healthcare Bio-Sciences Corp., Piscataway, NJ), equipped with a 14.0-MHz-probe. Measurements, averaged from ten consecutive cycles, included LV long- and short-axis diameters at end-diastole and end-systole, as well as posterior-wall thickness at end-diastole. Posterior-wall shortening velocity was measured

from M-mode recordings (guided by two-dimensional images), obtained at the mid-LV level.

The analysis was performed by two experienced operators (A. D. V. and P. M.), and was thoroughly reviewed by a third (C. P.), all blinded to group assignment. Ejection fraction (EF) was calculated using the Simpson's principle, whereas the sphericity index was defined as the quotient of short-to-long axis. Wall tension index (WTI), a measure of wall-stress, was defined as LV end-diastolic diameter divided by twice the posterior-wall end-diastolic thickness (Pantos et al., 2008).

Immunohistochemical analysis

Under sevoflurane-anesthesia, the animals were sacrificed with intra-cardiac KCl injection, which arrests the heart in diastole; the heart was excised and fixed in 10% neutral-buffered formalin for histological analysis. The samples (control: $n = 5$, alginate: $n = 4$, alginate + GH: $n = 4$) were embedded in paraffin and were cut axially in 4 μ m-sections; they were rehydrated and stained with hematoxylin/eosin for gross morphological evaluation. The length and thickness of the infarcted area were measured after Heidenhain's AZAN trichrome staining, whereas collagen-content was measured after sirius-red staining.

Additional sections were used for immunohistochemical analysis with the following antibodies: Monoclonal anti- α -smooth-muscle-actin (Clone 1A4, A2547, Sigma Aldrich) for *myofibroblast* detection. Monoclonal anti-ED1 antibody for *macrophage* detection (kindly provided by Professor C. Dijkstra, VU Medical Center, Amsterdam, The Netherlands); the latter staining was enhanced by antigen retrieval (10 min microwave boiling in 1 mM EDTA, at pH = 8) and avidin/biotin complex (Vector Laboratories Inc., Burlingame, CA). Blood vessels were stained with *Griffonia simplicifolia* BSI-B4 isolectin (L2140, Sigma Aldrich) and *angiogenesis* was quantified by counting the total number of vessels in the infarct and peri-infarct areas. Section images were obtained with a camera (DFC490, Leica Microsystems Ltd, Milton Keynes, UK) and were analyzed with QWin 3.1 (Leica). Measurements were obtained from 20 infarct and peri-infarct fields per sample, and were normalized for the total area of the tissue examined. All measurements and analyses were performed by an experienced operator (E. P. D.), blinded to treatment identity.

Statistical analysis

Values are reported as mean $\pm$ standard error of the mean. Continuous variables were normally distributed, as per the Kolmogorov–Smirnov test; hence, differences between two variables were compared with Student's *t*-test, whereas differences between three or more variables were compared with the analysis of variance, followed by *post hoc* Duncan's multi-stage test. Statistical significance was defined at an alpha value of 0.05.

Results

In vitro alginate-degradation

The spectra displayed an absorption-band between 240 and 300 nm, peaking at ~ 250 nm (Figure 1A), with increasing

Figure 1. *In vitro* properties of alginate-hydrogel. Photometric spectra of alginate-hydrogel (A). Degradation (B) displayed an initial steep curve (i), followed by a flattened curve for 4 days (ii) and a plateau for 3 days (iii). GH-release from the alginate-hydrogel (C) occurred mostly during the initial 24 h (i), followed by a plateau for 3 days (ii).

intensity over time. Alginate-hydrogel displayed an initial steep degradation curve, with $\sim 50\%$ of the maximal concentration observed at 24 h; this was followed by smooth rates for 4 days and, finally, by a plateau-phase for 3 days. The curve indicates almost complete degradation *in vitro* at 8 days (Figure 1B).

In vitro GH-release

As seen in Figure 1C, $\sim 70\%$ of GH was released from the alginate-hydrogel within 24 h; this was followed by a plateau, with completion of GH-release at 3 days.

In vivo alginate-degradation

The characteristic features of the biotin-alginate spectra were shoulders at 1680 and 1545 cm^{-1} , with a peak at 1267 cm^{-1} . Biotin-alginate (Figure 2) was abundantly present 3 days after injection ($77.8 \pm 4.1\%$ of the examined area), but only scarcely at 7 days ($2.0 \pm 0.2\%$), with nearly complete degradation at 15 days ($0.7 \pm 0.1\%$).

Absence of systemic effects after local GH

Between the beginning and the end of the study, the body-weight gain after alginate+GH was $2.3 \pm 0.7\%$ (of the initial body weight), which was comparable to the $3.4 \pm 1.1\%$ observed in the remaining animals, not treated with GH.

Echocardiographic indices of LV remodeling and function

Left ventricular (LV) end-diastolic diameter was shorter in the alginate + GH group ($5.61 \pm 0.35\text{ mm}$), when compared to controls ($7.92 \pm 0.34\text{ mm}$, $p < 0.001$), or to alginate alone ($7.21 \pm 0.33\text{ mm}$, $p = 0.0065$); interestingly, values were similar to those seen in sham-operated animals ($5.05 \pm 0.32\text{ mm}$).

End-systolic diameter in the alginate + GH group ($3.12 \pm 0.21\text{ mm}$) was comparable to sham-operated animals ($2.55 \pm 0.37\text{ mm}$), and shorter than in controls ($6.28 \pm 0.48\text{ mm}$, $p < 0.001$), or alginate alone ($4.58 \pm 0.38\text{ mm}$, $p = 0.020$). Representative examples of M-mode recordings are depicted in Figure 3(A).

The sphericity index (Figure 3B) after alginate + GH (0.317 ± 0.017) was almost identical to that observed in sham-operated animals (0.312 ± 0.025) and improved compared to controls (0.598 ± 0.019 , $p < 0.001$) or alginate alone (0.406 ± 0.015 , $p = 0.0043$).

As expected, EF was lower in the MI-groups (Figure 3C), when compared to sham-operated animals ($69.4 \pm 1.9\%$). Between the MI-groups, there was a trend ($p = 0.067$) towards higher EF in the alginate + GH group ($50.7 \pm 1.5\%$) compared to alginate ($42.1 \pm 2.5\%$), although values were comparable to controls ($44.0 \pm 4.3\%$). WTI (Figure 3D) was lower after alginate + GH (1.436 ± 0.106), compared to controls (1.951 ± 0.174 , $p = 0.0083$) or alginate alone (2.052 ± 0.093 , $p = 0.0029$).

Figure 2. Biotin-labeling of alginate. Examples of microscopic images (scale: 20 μ m), 3 (A), 7 (B) and 15 days (C) after biotin-labeled alginate intra-myocardial injection. A control-specimen (saline-injection) is shown in (D).

Figure 3. Left ventricular dimensions and shape. Examples from echocardiographic M-mode recordings (upper-panel), showing left ventricular end-diastolic and end-systolic diameters. Sphericity (left-panel), ejection fraction (EF, middle-panel) and wall tension index (WTI, right-panel) in the four groups; note the improved values after alginate + growth hormone (GH). Asterisks denote statistically significant differences.

Histologic indices of LV remodeling

The collagen-content in the infarct and peri-infarct areas was comparable in the three MI-groups (control: $32.3 \pm 6.9\%$, alginate: $36.2 \pm 8.7\%$, alginate + GH: $32.6 \pm 6.3\%$). Similarly, no significant variance was observed in the length of the infarct-area (as percent of septal-length). In contrast, the

thickness of the infarct-area (relative to the non-infarct-zone) was higher after alginate alone (0.41 ± 0.04) compared to controls (0.17 ± 0.02 , $p = 0.013$). Improved results were seen in the alginate + GH group (0.72 ± 0.06), in which infarct-thickness was higher compared to controls ($p < 0.001$) or alginate ($p = 0.0029$). Representative examples are shown in Figure 4.

Figure 4. Infarct-thickness. Examples of the infarct-area thickness after Heidenhain's AZAN-trichrome staining (scale: 500 μ m) from slices at the mid-ventricular level, with the right ventricle on the left hand-side. Note the thicker infarct-wall after alginate + growth hormone (GH).

Figure 5. Myofibroblast activation. Examples of α -smooth-muscle-actin staining for myofibroblasts (arrows) in the peri-infarct-area (scale: 50 μ m); note the higher myofibroblast-density after alginate + growth hormone (GH).

Inflammatory response

Inflammation was generally of low-intensity in all groups, although slightly more prominent after alginate or alginate + GH injections; nonetheless, no significant variance was present between groups.

Myofibroblast-count

Treatment with alginate + GH was associated with increased presence of myofibroblasts in the infarct-area; specifically, α -smooth-muscle-actin positive staining was higher in the alginate + GH group ($6.25 \pm 1.28\%$), compared to controls ($0.77 \pm 0.29\%$, $p = 0.013$), albeit only marginally higher ($p = 0.069$) compared to alginate alone ($2.74 \pm 1.22\%$). Representative examples of myofibroblast-counts are shown in Figure 5.

Angiogenesis

Microvascular-density in the infarct-area was higher after alginate + GH ($6.93 \pm 0.55\%$), compared to controls ($3.61 \pm 0.44\%$, $p = 0.0016$) or alginate alone ($3.76 \pm 0.44\%$, $p = 0.0018$). Representative examples are shown in Figure 6.

Discussion

Chronic heart failure following MI portends an adverse prognosis; therefore, the prevention of LV remodeling constitutes an important therapeutic target (Agathopoulos & Kolettis, 2014). Growth factors hold a therapeutic potential in this regard (O'Sullivan et al., 2011), calling for further evaluation on their sustained administration. Here, we examined the effects of GH, currently attracting

considerable research efforts (Cittadini et al., 1997, 2003, 1996; Elaiopoulos et al., 2007; Jayasankar et al., 2004; Mitsi et al., 2006), administered locally via an alginate-hydrogel.

Degradation of alginate-hydrogel and GH-release

In our *in vitro* experiments, alginate-hydrogel displayed an initial steep degradation-curve for 24 h, yielding prompt, sustained GH-release during this time-frame. This was deemed necessary, given the early onset of the infarct-expansion process and the previously demonstrated beneficial effects of timely initiation of GH-treatment (Mitsi et al., 2006; O'Sullivan et al., 2011). A second phase, characterized by slower degradation, was observed for a period of 4 days, followed by a 3-day plateau, leading to near-complete degradation at 8 days. These curves, reiterated in our biotin-labeled experiments, ensure biomaterial action during the critical infarct-expansion stage, early post-MI.

Alginate + GH

Our protocol-design enabled comparison of the alginate + GH biomaterial-construct not only with controls, but also with alginate alone, given its previously demonstrated beneficial effects on post-MI LV dimensions and shape (Landa et al., 2008). We report additional benefit from local, sustained GH-administration via the alginate-scaffold, supporting our hypothesis on favorable effects of prolonged GH-treatment on LV remodeling (Jayasankar et al., 2004). Body weight was normal after alginate + GH, indicating the absence of systemic effects. Our findings underscore the dual-role of injectable alginate-scaffolds as drug-delivery vehicles and tissue-engineered constructs.

Figure 6. Angiogenesis. Examples of angiogenesis in the peri-infarct-area (scale: 50 μ m); note the higher microvascular-density after alginate + growth hormone (GH).

Infarct thickness and wall-stress

Infarct thickness was increased after alginate, but it was further improved after alginate + GH, indicating additive effects of both treatment-elements. Our results are in agreement with those reported after sustained release of IGF-1 (followed by hepatocyte growth factor) from an alginate-scaffold; intra-myocardial injection of this construct in rats attenuated infarct expansion and preserved scar-thickness, mediated by decreased apoptosis and increased stem-cell recruitment in the peri-infarct area (Ruvinov et al., 2011). Our echocardiographic data expand these observations and indicate that the favorable morphologic changes in the infarct and peri-infarct areas improve global LV architecture, mediated by reduced wall-stress.

According to LaPlace's law, ventricular-wall thinning elevates wall-stress, which constitutes a powerful stimulus for topographic alterations in the infarct and non-infarct zones (Sutton & Sharpe, 2000); in fact, wall-stress correlates strongly with the development of LV remodeling in patients (Aikawa et al., 2001). The improved WTI, observed 3 weeks after alginate + GH in our experiments, corroborates the importance of this mechanism on global LV shape and function.

Inflammatory response

Characterization of the inflammatory/foreign-body response represents an integral part of tissue-engineering research (Anderson et al., 2008); however, there is relative scarcity of relevant data after myocardial alginate-implantation, despite its widespread use. Examined three weeks post-MI, inflammation (in the infarct and peri-infarct areas) was generally mild and similar in the three treatment-groups of our study, although a trend was noted towards more intense response after alginate or alginate + GH injections. Our experiments do not provide information on earlier time-points, but previous *in vitro* (Yang & Jones, 2009) and *in vivo* (Robitaille et al., 2005) evaluation of alginate suggested prominent early macrophage-activation, with subsequent rapid decline. This inflammation pattern is considered beneficial (Frangogiannis, 2014) and likely contributes to the salutary effects of biomaterial implantation, via the enhancement of inherent healing and regeneration responses (de Jong et al., 2014; Wright et al., 2012).

The role of myofibroblasts

An interesting observation in our study was the higher myofibroblast-count (in the infarct and peri-infarct areas)

following alginate + GH administration. This finding is in accordance with *in vitro* studies reporting fibroblast growth and proliferation by GH (Cook et al., 1988; Lee et al., 2010), and provides further explanation for the increased infarct-thickness in this group. This statement is reinforced by the previously demonstrated inverse relationship between LV dilatation and myofibroblast-count in the infarct-area (van den Borne et al., 2009). The mechanisms underlying the favorable effects of myofibroblasts are thought to involve changes in cellular proliferation and migration, modulation of extracellular matrix metabolism (Porter & Turner, 2009) and enhanced angiogenesis by secretion of vascular endothelial growth factor (Chintalgattu et al., 2003), leading to improved healing (Daskalopoulos et al., 2014) and attenuated infarct-expansion (Laeremans et al., 2011).

Of note, the higher myofibroblast-count, observed after alginate + GH, was not accompanied by increased collagen-content. This finding is in agreement with previous reports (Bruehl & Oxlund, 1999; Cittadini et al., 2003; Tanaka et al., 1998), and is beneficial, as excessive fibrosis affects LV relaxation and promotes arrhythmogenesis (Kolettis, 2006). Nonetheless, our present results should be viewed as preliminary, necessitating further research on the effects of GH on myofibroblast-activation.

Angiogenesis

The pro-angiogenic actions of GH were demonstrated long-ago (Struman et al., 1999), and were subsequently confirmed post-MI, after local administration in the porcine-model (Mitsi et al., 2006). The generation of newly formed blood vessels in this setting is important, because better perfusion accelerates infarct-healing processes (Cochain et al., 2013). Thus, in line with previous reports (Banquet et al., 2011; Hao et al., 2007; Ruvinov et al., 2011), the enhanced angiogenesis (observed after alginate + GH in our experiments) presents as an additional mechanism for improved morphology of the infarct-area.

Strengths and limitations of the study

We evaluated the sustained GH-release via a biomaterial-scaffold, which resulted in additive effects of both elements. This approach was implemented immediately post-MI, favoring the emerging concept targeting the *prevention* of topographic LV alterations, as opposed to their *reversal* in established heart failure; in the clinical setting, such treatments may be combined with revascularization procedures,

should this approach surface into clinical trials. The inclusion of *in vivo* functional data in our experiments, along with morphological end-points, adds value to the study; in addition, we included only male rats in our experiments, thereby avoiding gender as a possible confounding factor. Finally, we focused on the intriguing effects of GH on angiogenesis and myofibroblast-activation. Despite these strengths, four limitations should be noted. First, due to the relatively small number of observations, the treatment-effects on mortality were not examined. Second, despite the reproducibility of infarct size elicited by the ligation procedure, additional echocardiographic studies (performed early post-ligation) would have strengthened our results. Third, the inclusion of a rat group treated with GH alone would have enabled more comprehensive assessment of anti-remodeling strategies. Finally, the 3-week time-frame post-ligation suffices for the evaluation of LV remodeling, but longer intervals would have permitted the assessment of its progression into chronic heart failure.

Conclusions

Local GH-administration via an alginate-scaffold attenuated LV remodeling and improved LV function in the rat MI-model, more effectively than alginate alone. These actions, secondary to preserved scar-thickness and reduced wall-stress, can be attributed to the beneficial actions of GH on infarct healing, mediated by enhanced angiogenesis and myofibroblast-activation. Local, sustained GH-administration constitutes a promising strategy for the prevention of post-MI LV remodeling. Further work is needed on the optimal duration of GH-treatment, including comparison between single-dose, versus short- and long-term sustained administration, the latter encompassing the entire remodeling period.

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Declaration of interest

The authors report no declarations of interest.

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