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BASIC PHARMACOLOGY

Dalteparin Sodium (Fragmin®) Administration Following Acute Infarction does not Affect Myocardial Perfusion and Function in Swine

Mickey Scheinowitz,[1,2] Yuval Heled,[1] Pierre Chouraqui,[3] Zvi Vered,[3] Yfat Hayardeni,[1] Arkady Kotlyar,[1] David Castel,[1] Shy Livschitz,[3] Vivian Barak,[4] Naphtali Savion,[5] and Michael Eldar[1]

[1]*The Neufeld Cardiac Research Institute, Sheba Medical Center, Tel-Hashomer 52621, Israel;* [2]*Department of Biomedical Engineering, Israel;* [3]*The Heart Institute, Tel Aviv University, Sheba Medical Center, Israel;* [4]*Sharet Institute, Hadasa Medical Center, Jerusalem, Israel;* [5]*Goldschleger Eye Research Institute, Israel*

***Summary. Background*: Heparin and low molecular weight heparin (LMWH) derivatives are considered angiogenic factors in ischemic and infarcted myocardium. However, the effect of dalteparin sodium (fragmin®, a LMWH derivative) on angiogenesis post infarction has not yet been investigated.**

***Purpose*. To assess the effect of systemically-administered dalteparin sodium on left ventricular perfusion and function during remodeling in swine subjected to acute microembolization infarction (MI).**

***Methods*. MI was induced in 12 anesthetized female pigs which randomly received dalteparin sodium (12,500U) or placebo through intra-peritoneal osmotic pump, for one week, beginning immediately after the MI. Myocardial perfusion and function were measured using radionuclear scan and echocardiography, respectively, at baseline, immediately post MI, and at 2 and 4 weeks post MI. Dobutamine stress echocardiography was performed 4 weeks post MI. Serum level of basic fibroblast growth factor (bFGF) was analyzed using a commercially available ELISA kit.**

***Results*. No differences were observed in myocardial perfusion at all time points following MI. No significant changes were observed in myocardial function and wall motion during the 4 weeks follow-up period, or after dobutamine administration. Serum bFGF did not change throughout the study period.**

***Conclusion*. Under the setting of the current experiment, one week of dalteparin sodium administration does not affect myocardial perfusion and function following acute infarction in swine, either at rest or following pharmacological stress.**

***Key Words*. dalteprin sodium, serum basic fibroblast growth factor, dobutamine stress echocardiography, perfusion imaging, swine**

Introduction

In the past decade it has been documented that heparin can potentiate collateral growth and improve myocardial perfusion following acute infarction [1,2,3]. Carall and colleagues, for example, have shown that heparin improved coronary collateral blood flow 4 weeks post ischemia in swine [1], while Unger et al. found similar results in a canine model of acute MI [2]. Clinically, heparin was shown to improve LV function and to increase collateral blood flow in post MI patients [3]. Combined therapy of heparin and exercise resulted in improved exercise capacity in patients with chronic ischemic heart disease [4]. These studies suggest that heparin triggers myocardial angiogenic response. One explanation for the interaction between heparin and angiogenesis relates to the fact that heparin increases the binding affinity of FGF to its receptor and thereby increasing the angiogenic potential of the related vascular bed [5].

In addition to heparin, similar angiogenic responses were shown with the use of low molecular weight heparin (LMWH) derivatives [6,7]. Studies on patients with myocardial ischemia have found that dalteparin sodium, a 5 kDa derivate of heparin, and enoxaparin, a 5.5 kDa heparin derivative, significantly improved myocardial function following exercise [6,7]. These studies show that LMWH derivatives may exert angiogenesis and thereby improve myocardial function. The present study was undertaken to assess the effect of dalteparin sodium on myocardial perfusion and function during the healing phase following acute infarction. We hypothesized that dalteparin sodium would improve myocardial perfusion and as a result, also myocardial function.

Address for correspondence: Dr. M. Scheinowitz, Ph.D., The Neufeld Cardiac Research Institute, Sheba Medical Center, Tel-Hashomer, 52621, Israel. Tel.: 972-3-5302614; Fax: 972-3-5351139; E-mail: mickeys@post.tau.ac.il

Methods

Animals. The animals were handled according to the standards of the American Association for Accreditation of Laboratory Animal Care (AAALAC), and following approval by the Institutional Committee for Laboratory Animals at Tel Aviv University. Twelve female pigs weighing 30 ± 5 kg (mean ± SD) were subjected to acute microembolization infarction and thereafter randomly divided into two groups: 5 animals received intraperitoneal infusion of dalteparin (experimental group) and 7 animals received albumin (control group).

Myocardial infarction

The pigs were premedicated with azaperone (1 mg/kg) intramusculary and diazepam (10 mg) intravenously. General anesthesia was induced with intravenous thiopental sodium (20 mg/kg) and maintained with inhaled halothane (2–2.5%). The animals were mechanically ventilated through an endotracheal tube. Left coronary artery catheterization was performed through the femoral artery (9F catheter, CR Bard) using the Seldinger technique. ECG and arterial blood pressure were continuously recorded using Nihon Kohden polygraph (Japan). After intravenous injection of 200 U of heparin, a coronary angioplasty balloon (2 or 2.5 mm, CR Bard) was advanced into the distal left anterior descending coronary artery or to a dominant distal diagonal branch. The balloon was inflated to 100 mm Hg for 30 s and 50 μl packed affigel blue beads (75–150 μm, Bio-Rad Laboratories), suspended in 0.5 ml saline solution, were injected through the distal lumen of the balloon and flushed with 35% sodium and 65% maglumine diatrizoates (Urografin, Scherring AG, Germany) to produce distal embolization. The animals were observed under general anesthesia for 3 h and then extubated after recovering from anesthesia.

Osmotic pumps

Immediately after the infarction was induced, 2 Alzet osmotic pumps (model 2ML1, Alza Corporation, USA) which were loaded earlier with 2 ml of dalteparin sodium (fragmin®, Pharmacia AB, Sweden), 12,500 U each, or 2 ml albumin (1 mg each), were installed in the peritoneal space. Dalteparin perfusion rate was approximately 10.5 μl/h (75U/h) per pump, maintained for one week.

Perfusion imaging

To evaluate regional and global myocardial perfusion, technetium-99m (Tc-99m) tetrofosmin was injected IV (12 mCi). A 10 minutes image was performed in the anterior oblique projection, 30 minutes post-injection, using a mobile gamma camera (Apex 215M, Elscint, Israel). Images were obtained, while the animals were in supine position, prior to MI, immediately post-MI, and repeated 2 and 4 weeks post-MI. Data was stored on magnetic disks for subsequent analysis by 2 methods. (A) Visual analysis: Images were projected on a computer screen and semi-quantitatively analyzed by an unbiased investigator. The left ventricle was divided into 5 segments; antero-septal, infero-septal, apical, infero-lateral, and antero-lateral. Tc-99m uptake was scored in each segment using a 5 point-scoring system where 0 = normal, 1 = mildly reduced, 2 = moderately reduced, 3 = severly reduced, and 4 = no Tc-99m uptake. (B) Quantitative analysis: Circumferential maximum count profiles of the myocardial distribution of Tc-99m tetrofosmin were obtained. Each point of these profiles represents the maximum count per pixel along a radius traversing the myocardium. The profile was automatically reconstructed by the computer, from the values of 60 radii spaced, at 6 degrees intervals.

Evaluation of cardiac performance

To evaluate regional and global LV performance, a parasternal short axis 2-D echocardiography (model 77020A echocardiograph, Hewlett Packard) was performed using a 3.5 MHz transducer under complete anesthesia, pre-MI, immediately post-MI, 1.5 hours post MI, and repeatedly 2 and 4 weeks post MI. Data was recorded on a Panasonic video machine, and images were evaluated quantitatively for LV end diastolic area (cm^2), LV cavitary area shortening (%), and LV circumferential systolic thickening (%). An attempt was made to visualize and analyze as many of the 16 segments as possible from the short axis views at 3 levels: Basal—mitral valve level; Mid—mid-papillary muscle level; Apex—as close as possible to the apex, on the basis of the recommendations of the American Society of Echocardiography [8]. All measurements were performed in the papillary and apical levels. Semiquantitative measurements included regional wall motion analysis that was also based on the American Society of Echocardiography recommendations [8]. The injured wall segments in the same two levels were determined based on wall motion abnormalities (normal = 0, hypokinesia = 1, akinesia = 2, dyskinesia = 3). Dobutamine stress echocardiography was performed 4 weeks post infarction, at a dose of 1, 2, 4, and 8 μg/kg/min, administered intravenously. Measurements were performed after steady state (in blood pressure and heart rate) was achieved.

Serum basic FGF (bFGF) levels

Venous blood samples were collected at baseline (pre MI), immediately post-MI, and repeatedly 2 and 4 weeks post MI. Blood was centrifuged, serum was collected and stored at −70°C until analyzed. bFGF was determined by a commercially available kit (Quantikine, USA), and using ELISA technique [9].

Statistical analysis

The ANOVA test was used to compare results obtained at the 4 time points and following different doses of dobutamine administration. Student *t*-test was used to

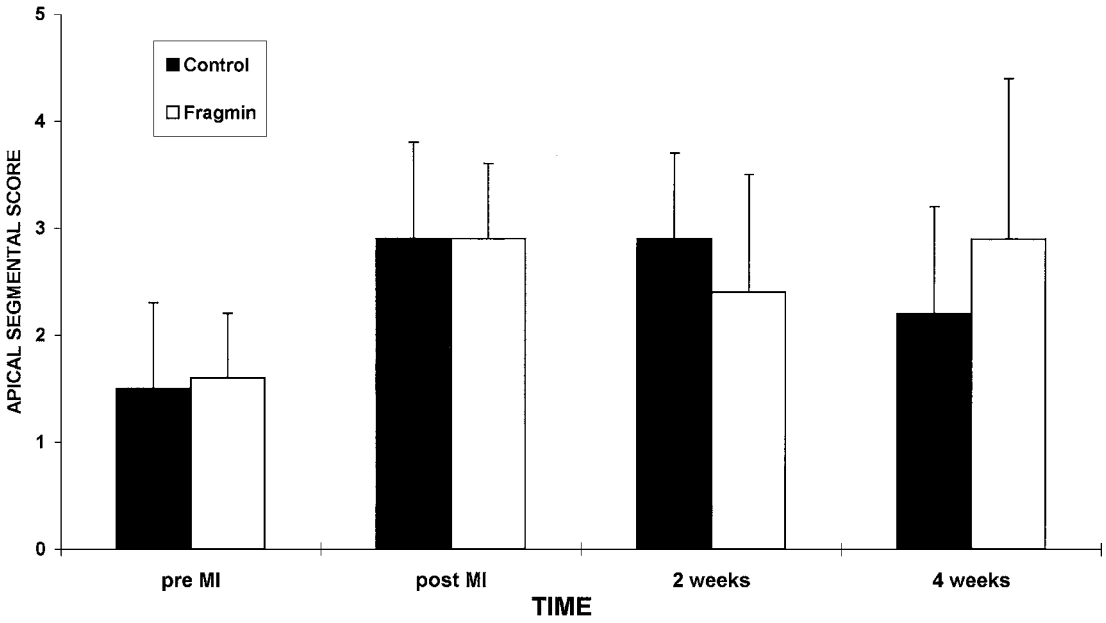

Fig. 1. *Visual analysis of myocardial perfusion at the apical level, pre MI, immediately post MI, 2 weeks and 4 weeks post MI.*

compare the results of dobutamine administration between the two groups of animals. Data are presented as mean ± SD. Values of $p \leq 0.05$ were considered statistically significant.

Results

Myocardial perfusion

Visual analysis (Fig. 1): As expected, a significant reduction in myocardial perfusion was demonstrated in the MI territory (apical segment) immediately after the infarction. No improvement in myocardial perfusion was observed along the 4 weeks follow-up, or between the treated and the control groups.

Quantitative analysis (Fig. 2) demonstrated a significant decrease in myocardial perfusion in the MI territory, in both groups, following the infarction (from 68.4 ± 3.0% pre MI to 56.9 ± 3.3, 49.6 ± 2.9, and 65.9 ± 9.5% post MI, 2 weeks and 4 weeks post MI, respectively, $p = 0.001$ in control group, and 71.8 ± 0.8, 46.9 ± 2.2, 60.6 ± 6.1, and 49.8 ± 3.7% for pre MI, post MI, 2 and 4 weeks post MI, respectively, $p = 0.001$ in the dalteparin group). No significant differences were observed, at all time points, between the experimental group and the controls.

Myocardial function

Regional wall motion was significantly reduced immediately post MI in both groups (Fig. 3(A)). There was a non-significant improvement in regional wall motion at 2 and 4 weeks post MI with no difference between the groups ($p = 0.48$). Dobutamine administration did not improve regional wall motion in both groups (Fig. 3(B)). Fractional LV cavitary shortening (Fig. 4(A)) was decreased following acute MI (6% in both groups) with complete restoration of cavitary shortening in the dalteparin treated group, and no change in control group. Nevertheless, no statistical difference was observed between the pattern of the two groups ($p = 0.09$). Dobutamine caused a significant dose response increase in LV cavitary shortening (from 46 ± 9% at baseline to 67 ± 13% during 8 μg dobutamine, and from 41 ± 2% to 61 ± 12% in dalteparin and control, respectively, $p < 0.001$), but with no difference between the groups ($p = 0.43$) (Fig. 4B).

Serum bFGF

Despite somewhat higher bFGF levels prior to MI in control group (37.4 ± 8.1 vs. 30.0 ± 18.3 μg/ml in dalteparin group), no difference was detected immediately after the infarction and during the 4-weeks follow-up within and between the 2 groups (Fig. 5).

Discussion

This study shows that one week of dalteparin infusion to swine subjected to acute microembolization infarction does not improve myocardial perfusion or function during the healing phase post infarction.

Myocardial infarction impairs LV function and can lead to death [10,11]. It is well known that the existence of coronary collateral blood vessels is an important parameter in minimizing myocardial damage secondary to acute infarction [12–14]. Angiogenesis has been shown to occur in the setting of myocardial ischemia [15,16] and following the administration of

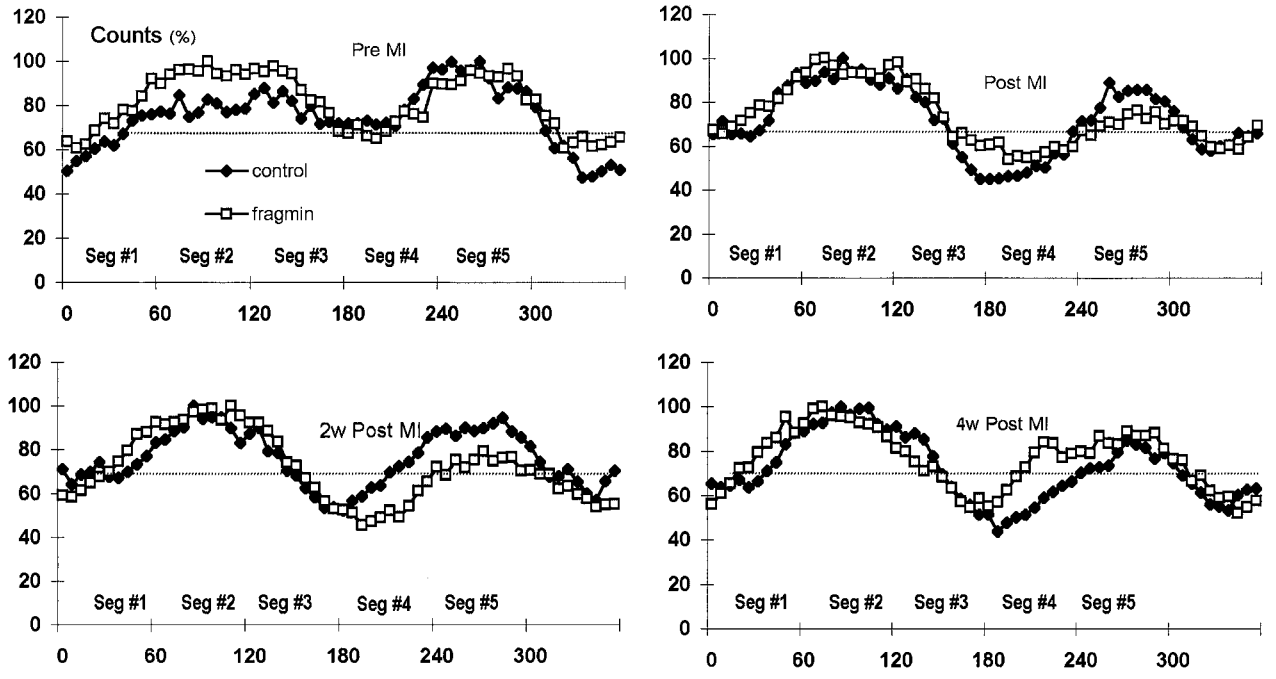

Fig. 2. Quantitative analysis of myocardial perfusion circumferential count profile of myocardial distribution of Tc-99m tetrofosmin, pre MI, immediately post MI, 2 weeks and 4 weeks post MI. Apical level is represented by segment number 4.

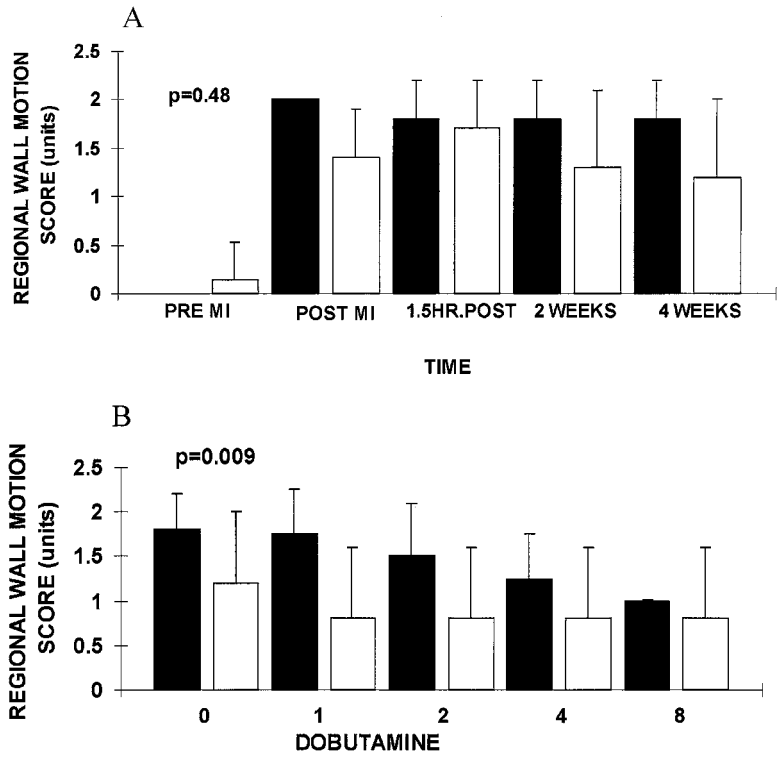

Fig. 3. Changes in regional wall motion score along the 4 weeks follow-up in fragmin (white bars) and control (black bars) groups (A), and following dobutamin administration (B). p = 0.009 represent dose-dependent differences within the two groups.

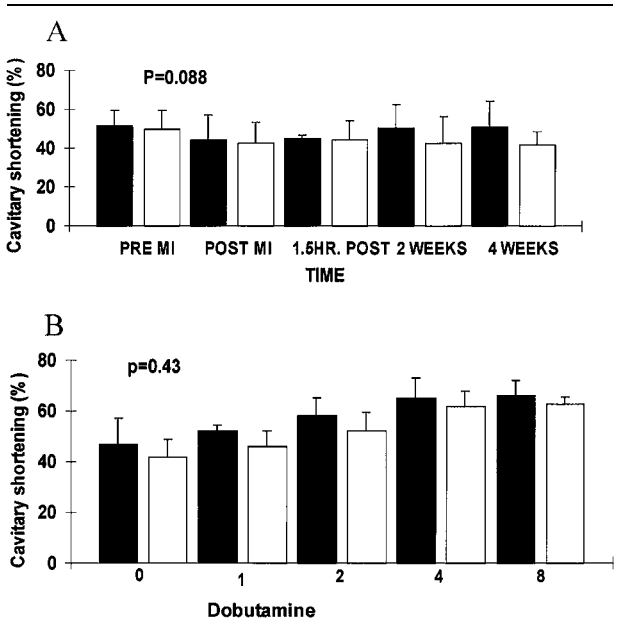

Fig. 4. *Changes in fractional LV cavitary shortening (%) along the 4 weeks follow-up in fragmin (white bars) and control (black bars) groups (A), and following dobutamin administration (B).*

heparin-binding growth factors such as bFGF [17,18] and vascular endothelial growth factor [19]. Previous studies have shown that heparin may potentiate bFGF expression and thus, enhance angiogenesis and improve myocardial perfusion [20] and function [1,2,3]. Clinically, repeated heparin administration was shown to reduce ischemia in chronic stable angina pectoris patients [21]. Other studies have suggested that LMWH derivatives such as dalteparin and enoxaparin may also potentiate angiogenesis and therefore improve myocardial perfusion or function following acute MI in human patients [6,7]. Quyyumi et al. [6] have shown that daily dalteparin injections (130 U/kg) for a period of 4 weeks, combined with exercise sessions, significantly decreased myocardial ischemia in patients with stable coronary artery disease. Fujita et al. [7] have shown similar results using enoxaparin (at a dose of 40–60 mg), combined with treadmill exercise for 3 weeks. Coronary catheterization demonstrated improved coronary collateral blood vessels in the enoxaparin treated patients. Similarly, Melandri et al. have shown improved exercise parameters following daily LMWH injections in patients with exercise-induced angina [22]. In another study, Fujita et al. have shown similar results following heparin administration to canine subjected to repetitive coronary artery occlusions [23]. In the present study 1 week of intraperitoneal dalteparin administration, at a dose of 60 U/kg, did not affect myocardial perfusion and function. One possible explanation may be related to the dose administered at the present study and that given by Quyyumi et al. to cardiac patients. While they injected 120 U/kg, we gave half the dose (60 U/kg). However, we delivered dalteparin continuously, which presumably kept a constant level in the blood for a week, rather than a single injection, daily.

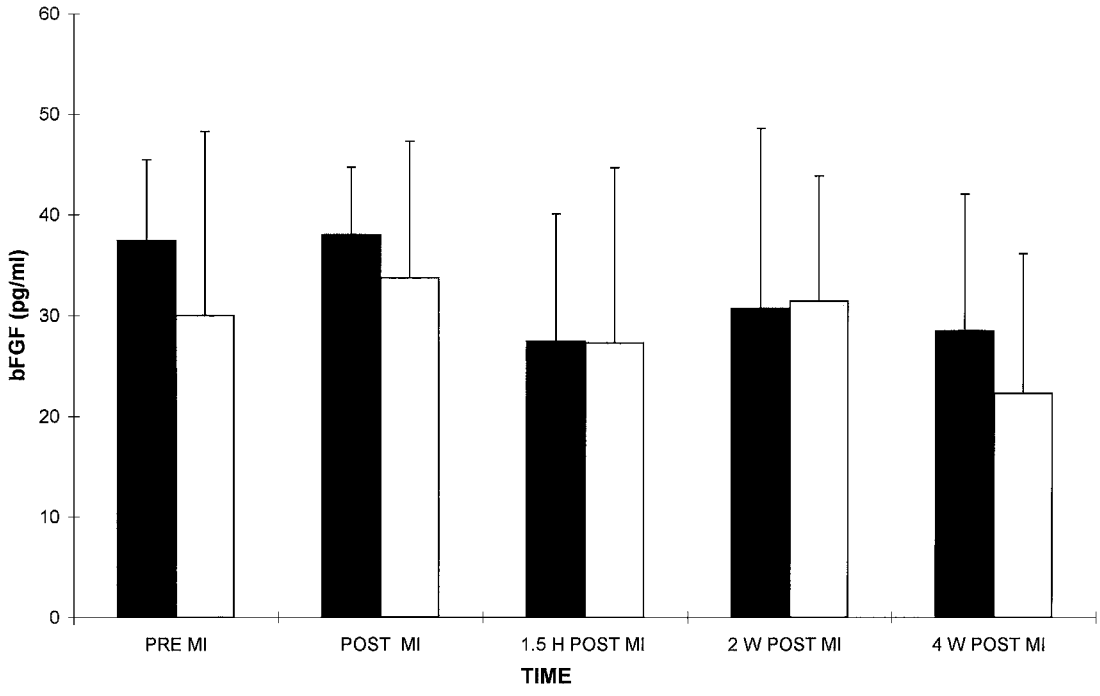

Fig. 5. *Changes in serum bFGF levels (pg/ml) in fragmin (white bars) and control (black bars) groups along the 4 weeks follow-up period.*

Another possible explanation may be related to the fact that in addition to the disease (myocardial ischemia versus myocardial infarction) the patients exercised on a daily basis in order to trigger coronary collateral growth [24]. The effect of additional stimuli (e.g. exercise) deserves further studies.

In the present study acute MI was induced by intracoronary administration of 75–150 μm beads thus, leading to microembolization infarction [17]. Although the pathophysiology of acute MI in a model of microembolization may be different from occlusion of a major coronary artery, it allows inducing controlled regional infarction in a chronic animal model. Myocardial perfusion was evaluated by successive radionuclide perfusion imaging studies during the 4 weeks follow up period based on the assumption that angiogenic processes after myocardial ischemia or acute infarction may be expected within this time frame [15,25]. In our study, dalteparin was administered into the peritoneum for one week using osmotic pumps, beginning immediately after the induction of the infarction. This time period of one week failed to induce angiogenesis and to improve myocardial perfusion following acute infarction. It is not known, however, whether different mode of administration and a longer delivery period would produce different result.

In the present study, myocardial function was evaluated by echocardiography during the 4-weeks post MI, at rest and following pharmacological stress, for the detection of hibernating myocardial segments in the injured myocardium [26]. Dalteparin treated animals did not demonstrate any improvement in regional myocardial function at all time points following the infarction. Induction of pharmacological stimuli 4 weeks post infarction demonstrated significant improvement in regional myocardial function in both groups, however, with no advantage of dalteparin over controls. Thus, under the setting of the present experiment, dalteparin does not exert cardioprotection following acute infarction.

Serum bFGF, a known marker for angiogenesis, was unchanged following the administration of dalteparin, throughout the 4 weeks post infarction period. Furthermore, it was not affected by the infarction immediately post and during the healing phase post infarction. Since there are contradictory reports regarding the effect of acute MI on serum bFGF levels [27–29], it is not surprising that dalteparin administration, under the setup of the present experiment, had no effect on this parameter.

Limitations

Several reasons might attribute to the lack of collateral formation/improved myocardial perfusion in our model. First, the use of microembolization infarction which dose not fully resemble the typical infarction obtain clinically in cardiac patients. Second, fragmin was administered only during the first week post infarction and thus no further stimuli for collateral growth was present from this time point on. Therefore, the results of the present study should imply to the model of microembolization infarction in swine, and cannot be extended to the clinical situation in patients with ischemic heart disease.

In conclusion, one week of intra-peritoneal administration of dalteparin sodium, a low molecular weight heparin derivative (dalteparin), had no effect on myocardial perfusion, myocardial function or serum bFGF levels following acute infarction in swine. The discrepancies between our data and that obtained from human patients deserve further studies.

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