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THE EFFECT OF INSULIN-LIKE GROWTH FACTOR-I AND II ADMINISTRATION ON MYOCARDIAL FUNCTION, PERFUSION AND STRUCTURE, IN A PIG MODEL OF MICROEMBOLIZATION INFARCTION

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I dedicate this work to all my relatives - those who can enjoy my happiness with me, and those who did not have an opportunity to live until this work was finished. It’s dedicated to my beloved grandfather Menashe Averbuh, who was the main family inspirer of coming to The Promised Land and did not reach the moment of achieving his dream, to my grandmother Miriam Averbuh who passed away in old age, to Dr. Yakov Zigelboim who was brutally killed, and others. I thank my beloved parents Gregory and Roza Kotlyar and my relatives Monik Zigelboim, Ada Shabat, and Shabtai Shabat for supporting me in all the meanings of the word.

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SUMMARY

Submitted by: Arkady-Avi Kotlyar

The Effect of Insulin-Like Growth Factor-I and II Administration on Myocardial Function, Perfusion and Structure, in a Pig Model of Microembolization Infarction

Supervisors: Dr. Mickey Scheinowitz, Prof. Michael Eldar,
Prof. Naphtali Savion

Background. Insulin-like growth factor (IGF) I and II are single chain polypeptides consisting of 70 (IGF-I) and 67 (IGF-II) amino acids. IGF-I and IGF-II are structurally and functionally related to each other and to insulin. The physiological source of IGF-I is in adult cells while this of IGF-II is in embryonic cells.

Both IGF's are acting via autocrine or paracrine pathways and are known to promote cell proliferation and differentiation in variety of tissues including cardiomyocytes. In several studies these growth factors were shown to induce cardiac hypertrophy *in vitro* and *in vivo*. Moreover, in studies that examined myocardial hypertrophy after myocardial infarction an increase in endogenous IGF's and its receptors was observed. The exogenous administration of IGF-I and IGF-II has been shown to improve cardiac function in rats following cardiomyopathy, ischemia and

reperfusion, cardiac failure and in pigs following myocardial infarction.

In addition, IGF-I and IGF-II were shown to prevent programmed cell death i.e. apoptosis in several types of tissues including cardiac myocytes. According to the ability of IGF-I and IGF-II to protect cells against apoptosis, the protective effect of exogenous administration of these growth factors after acute myocardial infarction might be expected.

None of the previous studies investigating IGF administration following myocardial infarction has studied the effect of these factors on myocardial perfusion. However, an angiogenic effect of exogenous IGF administration following acute myocardial infarction may be expected.

Methods. Twenty-one female pigs were subjected to acute myocardial infarction (MI) using distal microembolization infarction model, and received IGF-I and II (7 pigs in each group) and 7 pigs received pig albumin and served as control group. Serum cardiac troponin I concentration of each animal was detected before, immediately and 4 hours after the induction of MI. Echocardiography and perfusion imaging were performed during the 4 weeks of follow up period of the study and under pharmacologic stress. Four weeks after induction of myocardial infarction the animals were sacrificed upon anesthesia. Samples from representative infarct area, border area, and normal area of the myocardium were quickly frozen and used to assess DNA fragmentation. Another set of myocardial samples from the same areas was cut, embedded in paraffin and stained for:

1) hemotoxylin and eosin (H&E); 2) contractile (actin) and cytoskeletal (desmin) proteins; 3) endothelial cells marker von Willebrand Factor (vWF).

Results. Resting myocardial function of the infarct related area of IGF treated animals significantly improved 4 weeks following the induction of MI ($p=0.01$). Following dobutamine administration regional myocardial wall motion significantly improved in IGF-I compared to IGF-II animals ($p=0.05$), both being better than control ($p=0.03$). Myocardial perfusion, heart rate and blood pressure were similar in all animals during the study. Treated animals had lower serum cardiac troponin I concentration ($p=0.001$), more actin in the border ($p=0.01$) and infarct ($p=0.0001$) areas, more desmin in the infarct area ($p=0.0002$) and reduced DNA laddering in the infarct area, compared to control ($p<0.05$). IGF groups had more blood vessels in the border ($p=0.04$) and infarct ($p=0.003$) areas than control.

Conclusion. Both IGFs improved resting and pharmacologically stressed myocardial function, which was associated with preservation of myocardial structure, increased blood vessel density in the infarcted and border myocardial areas.

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ABBREVIATIONS

CK – creatine kinase

CK-MB – creatine kinase isoenzyme

cTnC – cardiac troponin C

cTnI – cardiac troponin I

cTnT – cardiac troponin T

DNA – deoxyribonucleic acid

DSE – dobutamine stress echocardiography

ECG – electrocardiogram

GH – growth hormone

H&E – hematoxylin and eosin

IGF – insulin-like growth factor

IGFBP – insulin-like growth factor binding protein

IGF-IR – insulin-like growth factor I receptor

IV – intravenously

LAD – left anterior descending

LV – left ventricle

MI – myocardial infarction

mRNA – messenger ribonucleic acid

PCR – polymerase chain reaction

RPM – revolutions per minute

SD – standard deviation

SE – standard error

VEGF – vascular endothelial growth factor

vWF – von Willebrand factor

ABSTRACT

Background. Insulin-like growth factor (IGF) I and II are single chain polypeptides consisting of 70 (IGF-I) and 67 (IGF-II) amino acids. IGF-I and IGF-II are structurally and functionally related to each other and to insulin. The physiological source of IGF-I is in adult cells while this of IGF-II is in embryonic cells.

Both IGF's are acting via autocrine or paracrine pathways and are known to promote cell proliferation and differentiation in a variety of tissues including cardiomyocytes. In several studies these growth factors were shown to induce cardiac hypertrophy *in vitro* and *in vivo*. Both IGF-I and IGF-II were shown to stimulate protein synthesis rate in adult rat cardiac myocytes, *in vitro*. A significant increase of IGF-I and IGF-II mRNA expression was observed in hypertrophied adult rat cardiomyocytes. Moreover, in studies that examined myocardial hypertrophy after myocardial infarction (MI) an increase in endogenous IGF's and its receptors was observed.

The exogenous administration of IGF-I and IGF-II has been shown to improve cardiac function in rats following cardiomyopathy, ischemia and reperfusion, cardiac failure and in pigs following MI.

In addition, IGF-I and IGF-II were shown to prevent programmed cell death i.e. apoptosis in several types of tissues including cardiac

myocytes. Myocardial cell death is found to occur in the heart following acute myocardial infarction and/or reperfusion as a result of both apoptosis and necrosis. According to the ability of IGF-I and IGF-II to protect cells against apoptosis the protective effect of exogenous administration of these growth factors after acute MI might be expected.

None of the previous studies investigating IGF administration following MI has studied the effect of these factors on myocardial perfusion. However, IGF-I and IGF-II were shown to induce angiogenesis directly and via regulation of Vascular Endothelial Growth Factor (VEGF) in several types of tissues. Therefore, an angiogenic effect of exogenous IGF administration following acute MI may be expected.

Methods. Twenty-one female pigs were subjected to acute MI using distal microembolization infarction model. Fourteen pigs received IGF-I and II (7 pigs in each group) and 7 pigs received pig albumin and served as control group. Venous blood samples were collected from each animal before, immediately and 4 hours after the induction of MI, in order to detect serum cardiac troponin I concentration. Echocardiography and perfusion imaging were performed during the 4 weeks of follow up period of the study in order to observe changes in myocardial function and perfusion, respectively, after IGF-I and IGF-II administration. In

addition, dobutamine stress echocardiography and perfusion imaging following adenosine infusion were performed 4 weeks post MI in order to study myocardial function and perfusion under pharmacologic stress. Four weeks after induction of MI the animals were sacrificed upon anesthesia. Samples from representative infarct area, border area, and normal area of the myocardium were quickly frozen in liquid nitrogen and afterwards stored at –80°C. Another set of myocardial samples from the same areas were cut and fixed in 4% buffered formaldehyde and afterwards embedded in paraffin. The frozen tissue samples were used to assess DNA fragmentation using DNA laddering technique. The paraffin-embedded myocardial samples were stained for: 1) hematoxylin and eosin (H&E); 2) contractile (actin) and cytoskeletal (desmin) proteins; 3) endothelial cells marker von Willebrand Factor (vWF).

Results. Myocardial function of all animals was normal at all levels before the induction of MI. The microembolization infarction caused a significant myocardial functional defect in all animals. No regional dysfunction following MI was observed at the basal parts of the heart. Myocardial function of the infarct related area of IGF treated animals significantly improved 4 weeks following the induction of MI ($p=0.01$). Following dobutamine administration regional myocardial wall motion significantly improved in IGF-I compared to IGF-II animals

(p=0.05), both being better than control (p=0.03). Before the induction of MI myocardial perfusion of all animals was within the normal range (Fig.14a). MI resulted in a significant myocardial perfusion defect in all animals. Myocardial perfusion remained similar in all animals at all time points during the follow-up period of the study. Heart rate and blood pressure were also similar in all animals during the study. IGF-I and II treated animals had lower serum cardiac troponin I concentration 4 hours post-MI (p=0.001). In addition, IGF groups had more blood vessels in the border (p=0.04) and infarct (p=0.003) areas than control. More actin in the border (p=0.01) and infarct (p=0.0001) areas, and more desmin in the infarct area (p=0.0002) were detected in IGF treated groups, compared to control group. Moreover, DNA laddering within the infarct area was reduced in IGF animals, compared to control ($p<0.05$).

Conclusion. Both IGFs improved myocardial function of infarcted animals at rest. Myocardial functional reserve of IGF-I treated animals was better than that of IGF-II, while in both it was better than in the non-treated group, as detected under pharmacologic stress. The improvement in myocardial function of IGF-I and II treated animals was associated with preservation of myocardial structure and increase in blood vessel density in the infarcted and border myocardial areas. The effect of IGF-I was more potent than IGF-II.

INTRODUCTION

Structure and function of Insulin-like growth factors

Insulin-like growth factor I (IGF-I) and II are single chain potent mitogenic and anabolic polypeptides consisting of 70 (IGF-I) and 67 (IGF-II) amino acids respectively. IGF-I and IGF-II are structurally and functionally related to each other and to insulin (Fig. 1) (1). The IGFs are produced by multiple body tissues under control of growth hormone (GH) and other regulatory factors (1,2). Adult cells are the physiological source of IGF-I while embryonic cells are that of IGF-II (2). The actions of the IGFs (as these of insulin) are mediated by cell surface receptors family. Namely, these are the highly homologous insulin receptor, Type 1 IGF receptor (IGF-I) and Type 2 IGF manose-6 phosphate receptor (IGF-II) (Fig. 2) (1,2). Both IGF-I and II act via autocrine, paracrine, and endocrine pathways and are known to promote cell proliferation and differentiation in variety of tissues including cardiomyocytes (1,2). In plasma and other body fluids, the IGFs are complexed to specific, structurally homologous binding proteins (IGFBPs) (3). Six IGFBPs have been cloned and sequenced in human and rat (3). The IGFBPs are numbered according to the sequence of their molecular characterization (3).

IGF-I

	1	10	20	30	40	50	60	70
HUMAN	GPETLCGAELVDALQFVCGDRGFYFNKPT	GYGSSSRRAPQT	GIVDECCFRSCDLRRLEMYCA	PLKPAKSA
PIG	----------	----------	----------	----------

IGF-II

	1	10	20	30	40	50	60	67
HUMAN	AYRPSETLCGGELVDTLQFVCGDRGFYF	SRPASRVSRRSR	GIVEECCFRSCDLALLETYCA	TPAKSE
PIG	----------	----------N---	----------	----------

Domain **B** **C** **A** **D**

Figure 1. Amino acid sequences of IGF-I and IGF-II. Dashed lines represent amino acids that are identical to human IGF. (1)

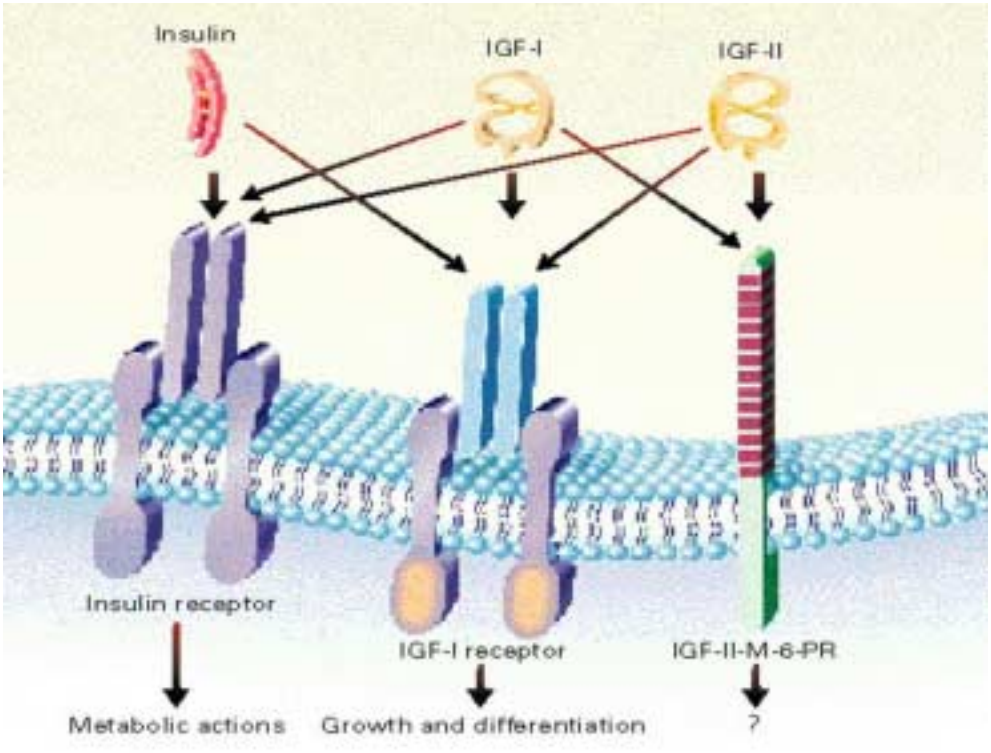

Figure 2. Insulin, IGF-I and IGF-II interactions with their receptors. The relative affinities of ligands for the various receptors are indicated by the width of the arrows; IGF-II-M-6-PR – manose –6-phosphate receptor family. (2)

Hypertrophic effect of IGFs on cardiac myocytes

In several studies these growth factors were shown to induce cardiac hypertrophy *in vitro* and *in vivo* (4-10). Moreover, in studies that examined myocardial hypertrophy after myocardial infarction an increase in endogenous IGF and their receptors was observed (11,12).

Ito and colleagues have shown that IGF-I directly stimulated cardiac hypertrophy in cultured neonatal rat cardiomyocytes (4). In another study, Adachi et al. have demonstrated a similar activity of IGF-II, that had a hypertrophic effect on cultured rat cardiomyocytes (5). In addition, Fuller and associates have shown both IGF-I and IGF-II to stimulate protein synthesis rate in adult rat cardiac myocytes, *in vitro* (6). A significant increase in IGF-I and IGF-II mRNA expression has been recently shown in hypertrophied adult rat cardiomyocytes. (7). Moreover, Czerwinski and coworkers have demonstrated that autocrine/paracrine stimulation of IGF-I gene may be involved in the mechanism of enhancing protein synthesis in response to increased workload in rats (9). Donohue et al. have shown that IGF-I may participate in initiating ventricular hypertrophy in response to altered loading conditions in spontaneously hypertensive rats (10).

In the model of acute myocardial infarction in rats an enhanced expression of IGF-I has been shown to be associated with the infarction and the mitotic division of remaining cardiac myocytes. (11,12).

IGFs and angiogenesis

Both IGFs are contributory to myocardial angiogenesis. IGF-I has been shown to be involved in inflammation-linked angiogenic process after microembolization induction in porcine heart (13). Kluge et al. have shown, that increased IGF-I mRNA expression occurred by infiltrating monocytes in areas of focal necrosis induced by microspheres, where capillary sprouting was detected (13). Moreover, elevated levels of IGF-I promoted neovascularization through increasing vascular endothelial growth factor (VEGF) gene expression in different models (14-16). In the work of Akagi et al. different types of human colon cancer cells were incubated in the presence of IGF-I for various time periods, which induced a VEGF mRNA expression (14). Similarly, in the work of Goad and colleagues, IGF-I stimulated VEGF mRNA levels in cultured osteoblast-like cells (15). Punglia et al. have shown that IGF-I-treated retinal pigment epithelial cells increased VEGF protein levels in conditioned media and stimulated capillary endothelial cell proliferation

(16). In addition, VEGF mRNA was increased in cultured human hepatoma cells incubated with IGF-II (17), and its direct angiogenic activity was observed in the quantitative chick chorioallanotic assay. (18).

Although, involvement of IGF in angiogenesis has been previously shown, none of the previous studies investigated the effect of IGF on myocardial perfusion *in vivo*.

Protective effects of IGFs administration on the heart

The beneficial effect of exogenous IGF administration was previously demonstrated in different animal models (19). Exogenous administration of IGF-I (20-22) and IGF-II (23,24), improved cardiac function in rats, following cardiomyopathy (20), ischemia and reperfusion (21), cardiac failure (22), and in pigs following myocardial infarction (23) and reperfusion (24). Ambler and colleagues have shown that subcutaneous injection of IGF-I performed weekly for 12 weeks improved the function of damaged myocardium (myocytes with disorganized myofibriles and necrotic infiltrate) in rats with established cardiomyopathy (20). Buerke et al. have shown that IGF-I administered 1h prior to ischemia, significantly attenuate myocardial injury in a rat

model of ischemia and reperfusion (21). Intraperitoneally administrated human recombinant IGF-I (0.5mg of IGF released for 1 week from implanted pump) did not have any effect on left ventricular geometry and infarct size in rat model of AMI (25). However, in another study, human recombinant IGF-I released for 14 days from intraperitoneally implanted pump, improved myocardial function of infarcted rats and caused myocardial hypertrophy (22). In humans, IGF-I was shown to improve cardiac function after myocardial infarction by stimulating contractility and promoting tissue remodeling (26).

To-date, only two studies have examined effects of IGF-II administration on MI (23,24). One of these studies was performed in our laboratory by Battler and coworkers (23). This work was performed using the model of microembolization myocardial infarction in pigs (23). The MI was induced by the intracoronary injection of affigel beads, which contained the factor, and were able to slowly release it (23). In this work IGF-II ameliorated regional myocardial function (23). The other work, performed by Vogt and colleagues, investigated intramyocardial infusion of IGF-II in swine, for 60 min prior to a 60 min LAD occlusion and 120 min reperfusion (24). In the recent work, myocardial infarction compared to the region at risk was significantly decreased by IGF-II treatment (24).

Constitutive overexpression of IGF-I positively influenced the performance of myocytes from transgenic mice by enhancing shortening velocity and cellular compliance (27), and prevented activation of cell death in the viable mice myocardium after infarction (28).

IGFs and cell death

In addition, IGF-I and IGF-II were shown to protect several types of tissues including cardiac myocytes from necrosis and programmed cell death i.e. apoptosis (8,21,29-34).

Myocardial cell death occurs in the heart as a result of both apoptosis and necrosis (Table 1), following acute myocardial infarction (35) and/or reperfusion (21).

Table 1. Degradative cell changes that follow necrotic and apoptotic types of cell death (35).

Necrosis	Apoptosis
swelling	shrinkage, chromatin condensation
blebbing and zeosis	budding
lysis	fragmentation
phagacytosis	phagocytosis by macrophages/nearby cells
inflamation	no inflamation

Buerke et al have shown in the rat model of myocardial ischemia and reperfusion, that IGF-I protected myocytes from leukocyte-induced cardiac necrosis and inhibited reperfusion induced apoptosis (21). In non-heart models IGF-I has been shown to prevent apoptosis trough IGF-I receptor (IGF-IR) in different cell types and tumors (30,31). However, circulating IGF-binding proteins (IGFBP) limit the access of the IGFs to specific tissues (2, 30). Some IGFBPs bind the growth factors with greater affinity than do the IGF receptors, thereby preventing the activation of intracellular signaling pathways (2, 30). As it was shown and schematically described by Rajah and associates in Figure 3, the independent and interdependent effects of IGFs and IGFBPs on the regulation of cell number involve two pathways that interact at several levels (30). IGFs mediate survival via the IGF receptor. IGFBP-3, for instance, is able to block this pathway by sequestering IGFs away from the IGF receptor. IGFBP-3 mediates apoptosis via its own receptors, while IGFs can prevent this effect by binding to IGFBP-3. Thus, IGFBP-3 can mediate cell death by both IGF-dependent and IGF-independent pathways. The survival signaling pathways from the IGF-IR are at least partially known (33,31). Sell et al and Resnicoff et al have demonstrated prevention of apoptosis by IGF-I receptor (IGF-IR) activation *in vitro* (32) and *in vivo* (33), in BALB/c

3T3 cells, and in several transplantable tumors from humans and rodents, respectively. In addition, IGF-II has been shown to prevent apoptosis in fetal human immune system (34).

According to the ability of IGF-I and IGF-II to protect cells against cell death, the protective effect of exogenous administration of these growth factors after acute MI may be expected.

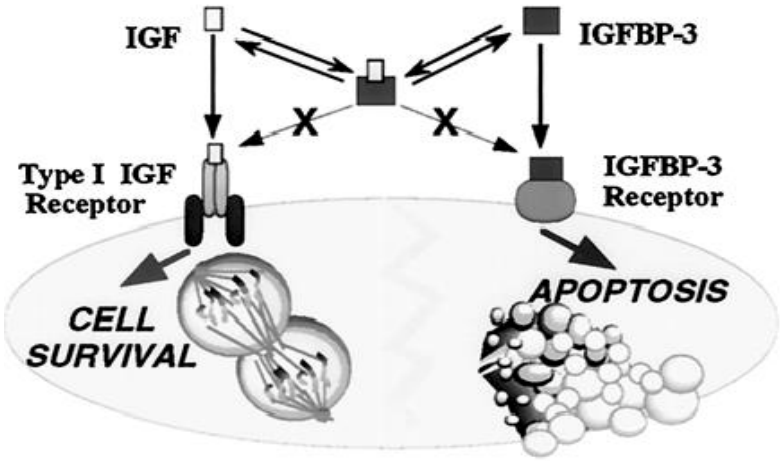

Figure 3. IGF induced prevention of apoptosis through the IGF-I receptor. IGFBP-3 – IGF binding protein 3 (30).

Serum markers of myocardial injury

Several serum biochemical markers are known to play an important role in diagnostics and evaluation of myocardial injury caused by ischemic heart disease and acute MI (36-38). Energy-producing proteins creatine kinase (CK) and myoglobin are upregulated following cellular damage in skeletal and myocardial muscle (36-38). In addition, structural and regulatory protein troponin, compartmentalized into minor

cytosolic and major myofibrillary bound fractions, is released into the circulation following myocardial cell injury and necrosis (36-38). Increased serum CK and its cardiac fraction CK-MB enzyme levels (activity) have been used for years to detect MI (36,37). However, it has been discovered recently, that serum myoglobin, CK-MB mass (concentration), or troponin T and I levels may indicate MI earlier (36,37). Cardiac troponin T and I (cTnT and cTnI) have been suggested as new, more specific markers of myocardial cellular damage (37,38). In the work performed in porcine hearts with severe left ventricular remodeling, Ricchiuti et al have demonstrated that the appearance of cTnT and cTnI in the blood is proportional to their chronic loss from injured myocardium (39). Although both cTnT and cTnI were proved to be strong, independent predictors of cardiac events, the sensitivity and accuracy of cTnI was found to be more reliable, when compared to cTnT (38). Moreover, cTnI appears to have statistically high diagnostic sensitivity and specificity for detection of myocardial injury during the first 48 hours of acute MI (37). Therefore, cTnI was used in the present work as a serum cardiac marker to investigate whether IGF administration protects myocardium following AMI. In the present study we investigated the relationship between IGF “therapy” and serum cardiac troponin I.

OBJECTIVES

IGF-I and II administration were previously shown to improve myocardial function in different animal models (19-21,23,24). According to the activities of IGFs we hypothesized that both factors preserve myocardial function following acute MI. We attempted to understand whether preservation of myocardial viability was related to preservation of cellular structure, decreased cell death and increased blood vessel density (Fig. 4).

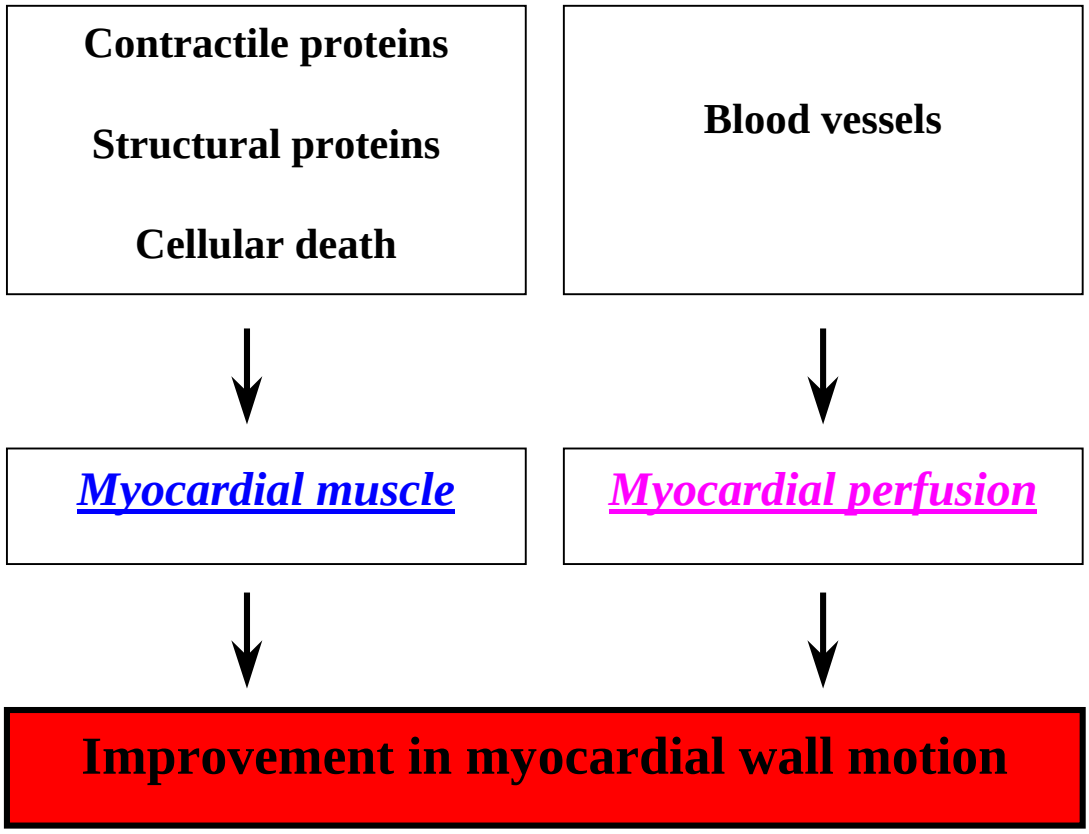

Figure 4. Possible mechanisms involved in the improvement of myocardial function.

We attempted to investigate which cytokine (adult characteristic - IGF-I or embryo characteristic IGF-II form) is more potent in preserving myocardial function after acute MI. Thus, a comparison of the effects of IGF-I and IGF-II within the same animal model of acute MI was a goal of the present work. Furthermore, the present study was designed to investigate through which possible mechanism the improvement in myocardial function following MI is being accepted (i.e. via affecting myocardial muscle and/or myocardial perfusion (Fig. 4)). Moreover, we have striven to investigate the effect of IGF-I & II on the above processes. Therefore, we investigated the effects of IGF-I and IGF-II following acute MI (in swine) on the following parameters:

1. myocardial function and perfusion;
2. serum cardiac troponin I concentration;
3. myocardial contractile and structural proteins;
4. blood vessel density;
5. DNA fragmentation;

The results of the present study may help to understand the role of IGF-I and IGF-II in preservation of myocardial structure and function following acute MI and may be considered as a potential therapy in cardiac patients in the future.

MATERIALS AND METHODS

Experimental protocol

The study was conducted under the supervision of a certified veterinarian, following the approval of the experimental protocol by the *Institutional Animal Care and Use Committee.* The animals were handled according to the *Guide for the Care and Use of Laboratory Animals,* published by the US National Institute of Health (NIH publication No 85-23, revised 1996).

Twenty-six female pigs (average weight 26 ± 2.5 kg) were subjected to acute MI using distal microembolization infarction model as described previously (23,40). Animals were randomly divided into three equal groups (finally 7 pigs in each group), which received IGF-I, IGF-II, and pig albumin incorporated within the intracoronary injected microspheres. After the induction of MI, the animals were followed up for 4 weeks. The growth factors were slowly released from the microspheres, which at the same time were used for the induction of acute MI. The period of 4 weeks post-MI was chosen as a time period during which the process of remodeling is completed in pig (41). In order to investigate myocardial function and perfusion after IGF-I and

IGF-II administration, echocardiographic functional study and perfusion analyses respectively, were performed during the four-week post-MI follow-up period. In order to investigate myocardial function and perfusion under pharmacologic stress, dobutamine stress echocardiography and perfusion imaging following adenosine infusion, respectively, were performed 4 weeks post-MI, before the euthanasia. Pharmacologic stress agents fall into two categories: cardiac positive inotropic agents such as dobutamine, and coronary vasodilating agents such as adenosine (42). Inotropic agents work indirectly by increasing myocardial workload, which then leads to an increase in coronary blood flow, whereas vasodilating agents work directly on the coronary vessels to increase blood flow (42). Bruce and associates reported that dobutamine has a similar effect on heart rate as exercise, but there is typically less of an increase in blood pressure (43). It is a synthetic catecholamine that increases wall stress, contractility, and cardiac work, predominantly through beta-adrenergic stimulation in a dose-dependent manner. There is some alpha1-2-adrenergic stimulation, and afterload is reduced by peripheral artery dilation. These effects, along with dobutamine's half-life of about 2 min, make it useful for cardiac stress testing. Low-dose dobutamine response can accurately predict dysfunctional yet viable myocardial regions. As shown by Travain and

colleagues, vasodilating agents are particularly advantageous in assessing post-myocardial infarction patients (42). Adenosine is a potent coronary vasodilator. Adenosine causes coronary vasodilation in most vascular beds (except in renal afferent arterioles and hepatic veins where it produces vasoconstriction) by acting directly on purine receptors (cell surface A1 and A2 adenosine receptors). Thus, in the coronary arteries adenosine is able to determine coronary vasodilatory reserve following MI. The exact mechanism by which adenosine receptor activation relaxes vascular smooth muscle is not known. There is evidence for both inihibition of the slow inward calcium current (reducing calcium uptake), and for activation of adenylate cyclase through A2 receptors in smooth muscle cells. This, in turn, relaxes the vascular smooth muscle and results in coronary arteriolar dilatation.

In addition, venous blood samples were collected from each animal before, immediately and 4 hours after the induction of MI.

Four weeks after induction of myocardial infarction the animals were sacrificed upon anesthesia. Samples from representative infarct area, border area, and normal area of the myocardium were quickly frozen in liquid nitrogen and afterwards stored at –80°C. Another set of myocardial samples from the same areas were cut and fixed in 4% buffered formaldehyde and afterwards embedded in paraffin. The frozen

tissue samples were used to assess DNA fragmentation using DNA laddering technique. The paraffin-embedded myocardial samples were stained for: 1) hematoxylin and eosin (H&E); 2) contractile (actin) and cytoskeletal (desmin) proteins; 3) endothelial cells marker von Willebrand Factor (vWF).

Induction of Acute Myocardial Infarction

The animals were premedicated with 2-3.5 mg/kg azaperone, intramuscularly. After intravenous (IV) administration of thiopental sodium (10-12 mg/kg) general anesthesia was performed by halothane 1.5-2% in oxygen inhaled through an endotracheal tube. Left coronary artery catheterization was performed via the femoral artery using the Seldinger technique (44). An over-the-wire intracoronary balloon angioplasty catheter (2.0 or 2.5 mm) was advanced into the distal left anterior descending coronary artery (1/3 away from the apex) followed by IV heparin (120U/kg) injection. After the balloon had been inflated to 6 ATM for 30 sec, 50 μl of affigel blue non bio-degradable beads (75-150 μm, Bio-Rad Laboratories, CA, USA) suspended in 0.5 ml of saline were injected via the distal lumen of the balloon catheter to induce distal myocardial microembolization infarction. Deflation of the balloon

was carried out 30 sec after the injection of the beads. Thus, the total time during which the balloon held inflated was about 60 sec. Blood pressure and peripheral/surface ECG were continuously monitored during the procedure using I, II, III, aVR, aVL and aVF leads (Fig. 5). The experimental groups received beads containing recombinant human IGF-I or IGF-II (150 μg / heart, Pharmacia AB, Sweden), while the control group received beads containing pig albumin (Sigma, St. Louis, MO) of same dosage. The affigel beads are characterized by their ability to slowly release the pre-bound growth factor (45). Thus the beads were used to induce acute MI and to provide a slow release delivery system of the peptide.

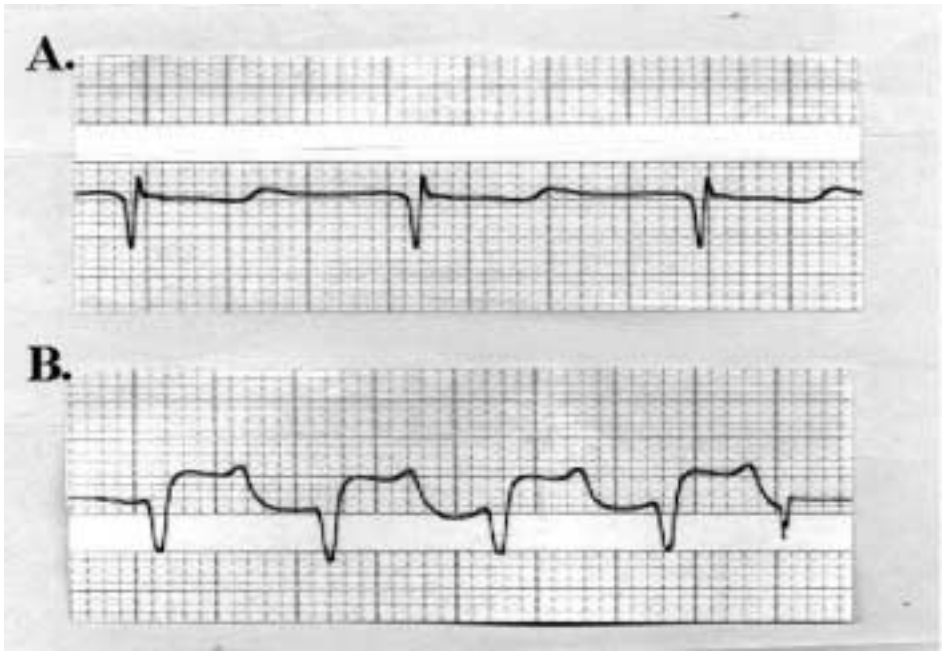

Figure 5. Representative section of ECG recording. A – pre-MI; B – immediately post-MI.

Assessment of myocardial function

In order to evaluate regional and global left ventricular function, parasternal long and short axis 2-D echocardiography (77020A Ultrasound, Hewlett Packard, Andover, MA, USA) was obtained under general anesthesia, pre-MI, immediately post-MI, 2 hours post, 2 weeks and 4 weeks post-MI. During the follow-up period all measurements were performed at the animals' steady state, after the animals have been anaesthetized and reached hemodynamic and respiratory stability. Four weeks post-MI echocardiography was obtained also during IV administration of dobutamine hydrochloride (Dobutrex, Lili GMBH, Giessen, Germany) (1, 2, 4 and 8 μg/kg/min) (46). Short axis echocardiography images were obtained at basal, mid papillary muscle and apical levels (47) (Fig 6A); recorded on ½ inch videotapes and analyzed by two unbiased investigators. Left ventricular (LV) cavity area, myocardial thickening and shortening were measured to assess LV global myocardial function. Images at each time period, at each level were visually divided into septal, anterior, lateral, posterior and inferior wall segments within the LV (Fig. 6B), based on the recommendation of the American Society of Echocardiography (47). Regional wall motion

of different LV segments was scored on the basis of wall motion abnormalities (mild hypokinesia, moderate hypokinesia and diskinesia or akinesia) (47). In this 3 point scoring system, the segmental wall motion is inversely related to the score and directly related to the damage in myocardial wall motion: mild hypokinesia = 1, diskinesia or akinesia = 3. Calculation of global LV function was based on the summation score of all segments for each animal, at each time period.

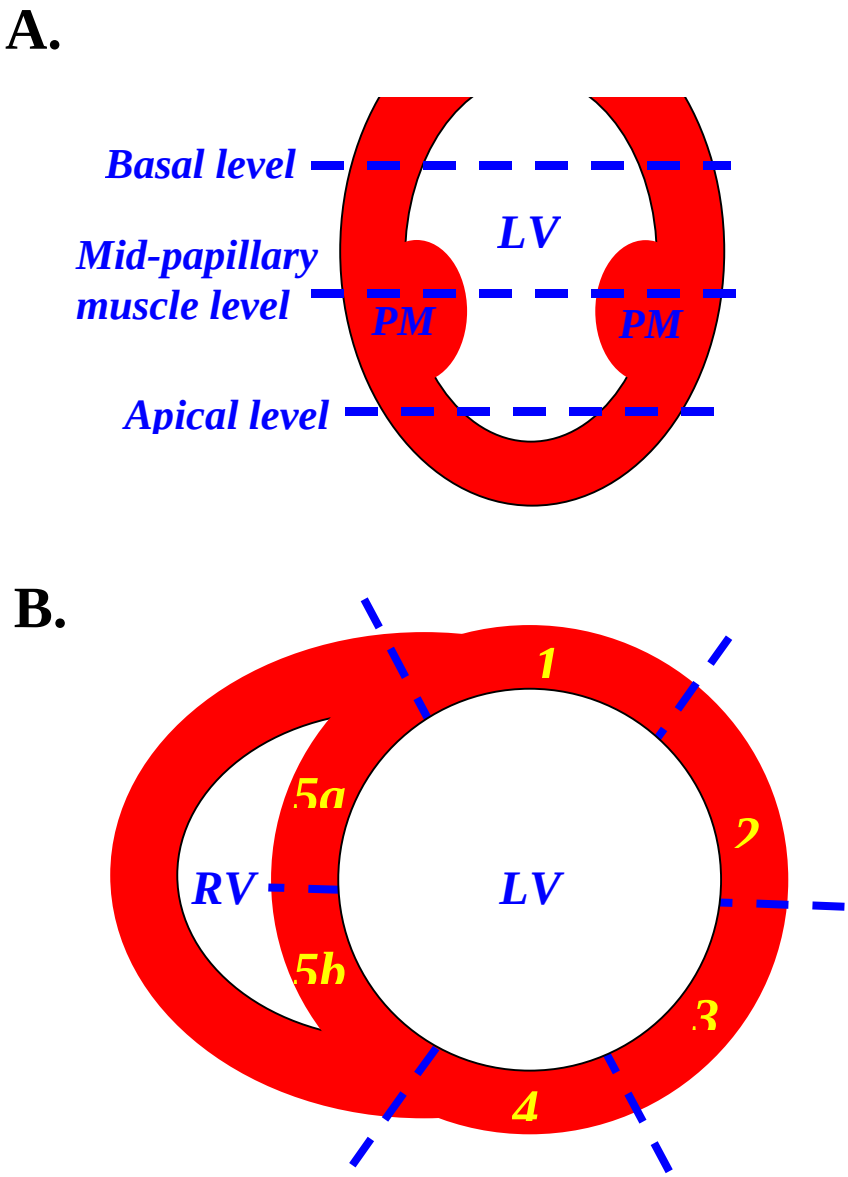

Figure 6: A. Scheme of left ventricle division by levels at long axis.

B. Scheme of left ventricle division by segments at short axis (apical view). LV – left ventricle; RV – right ventricle; PM – papillary muscle; 1 – anterior wall; 2 – lateral wall; 3- posterior wall; 4 – inferior wall; 5a – anterior septum; 5b – posterior septum.

Assessment of myocardial perfusion

Nuclear scanning with Technetium was used to evaluate regional and global LV perfusion. Technetium-99m tetrofosmin was injected IV (12 mCi). A 10-minute image was performed in the left anterior oblique projection, 30 minutes post-injection. Using a mobile gamma camera (Apex 215M, Elscint, Israel), imaging was performed pre-MI, immediately post-MI, 2 and 4 weeks post-MI. Four weeks post-MI, myocardial perfusion was measured also following IV adenosine (9-β-D-Ribofuranosyladenin, Sigma, St. Luis, MO, USA) administration. Mean systemic blood pressure in the femoral artery was monitored and recorded during adenosine administration, using Model 60-800 Physiologic Pressure Transducer (Trantec®, Baxter Healthcare Corporation, Uden-Holland) connected to Polygraph System (Nihon Kohden, Japan). Adenosine was administered until 30% reduction in mean systolic blood pressure was recorded (48) (Fig. 7). Data was stored on magnetic disks for subsequent analyses. Perfusion images obtained at each time period were projected on a computer screen and visually divided into septal, anterior, lateral, posterior and inferior LV wall segments (49). An unbiased investigator qualitatively assessed the

perfusion of each LV segment, according to standard technique (49). In this 5 point scoring system, the segmental perfusion is inversely related to the score and directly related to the damage in myocardial perfusion: normal = 0.

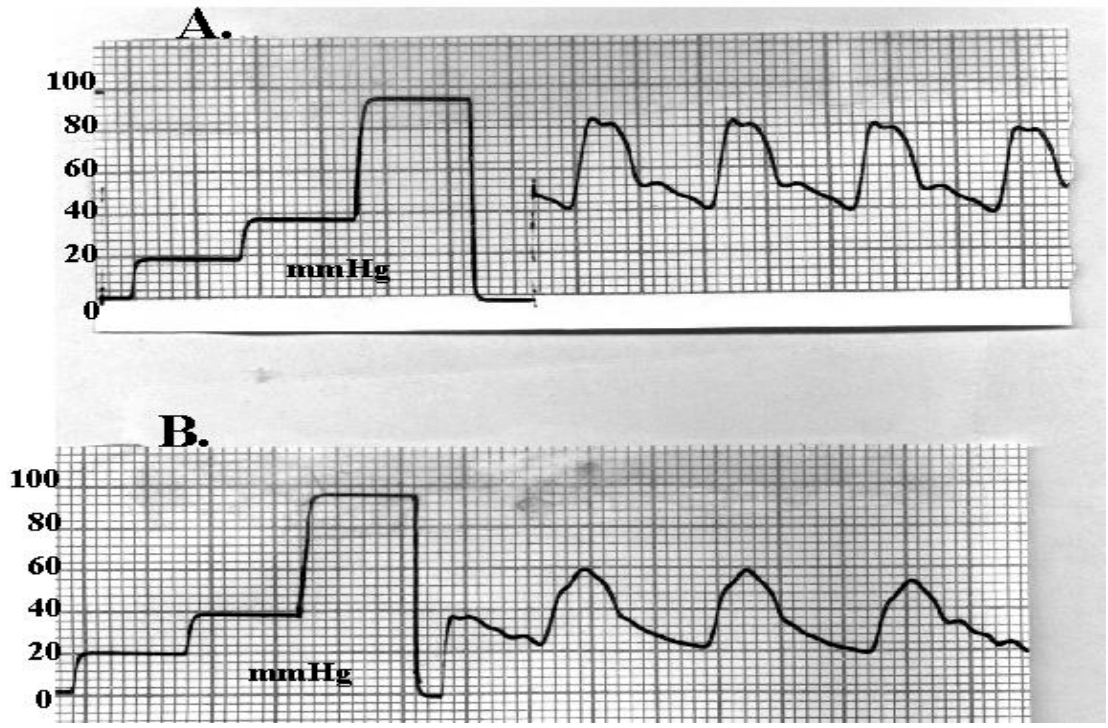

Figure 7. Representative section of blood pressure recording. A – before adenosine infusion (80 mmHg); B – after adenosine infusion (60 mmHg).

Evaluation of serum cardiac troponin I concentration

For serum cardiac troponin I evaluation, venous blood samples from each animal were collected at baseline, immediately post-MI and 4 hours post-MI. The samples were incubated for 20 min and then centrifuged for 5min at 3000 rpm, (Sigma 2 GmbH laboratory centrifuge, Western Germany), and the separated serum of each animal was frozen at –80°C. The microparticle enzyme immunoassay was used to determine serum cardiac troponin I concentration (50). The quantitative determination was performed on the AxSYM system (Abbot Diagnostics, USA), using AxSYM Troponin I Reagent Pack for in vitro diagnostic use. Serum cardiac troponin I concentration determined for apparently healthy human individuals in the AxSYM Troponin I assay is ≤ 0.4 ng/ml, while the diagnostic cutoff for the acute MI patients is approximately 2.0 ng/ml (50). To-date there is no data determining diagnostic limits of serum troponin I concentration in pig and the effects of IGF-I and II were yet to be investigated.

Euthanasia and myocardial processing

Four weeks post-infarction, the animals were reanesthetized and myocardial function and perfusion were measured at rest and following pharmacologic stress as previously described. Thereafter the chest was opened and the heart was excised and removed. The infarcted myocardial region was visually determined by the presence of fibrotic tissue and decreased LV wall thickness (Fig. 8). Two transverse sections including the infarcted myocardial region (approximately 1cm in width) were immediately cut. Two sets of histological samples from each representative MI area were collected from infarct, border and normal areas (Fig. 8). Samples of one set were quickly weighed and frozen in liquid nitrogen (approximately within 20 sec) while the other set was preserved in 4% buffered formaldehyde and after that paraffin-embedded. The frozen tissue samples were used for biochemical analyses, while paraffin-embedded myocardial samples were used for histopathological and immunohistochemical analyses.

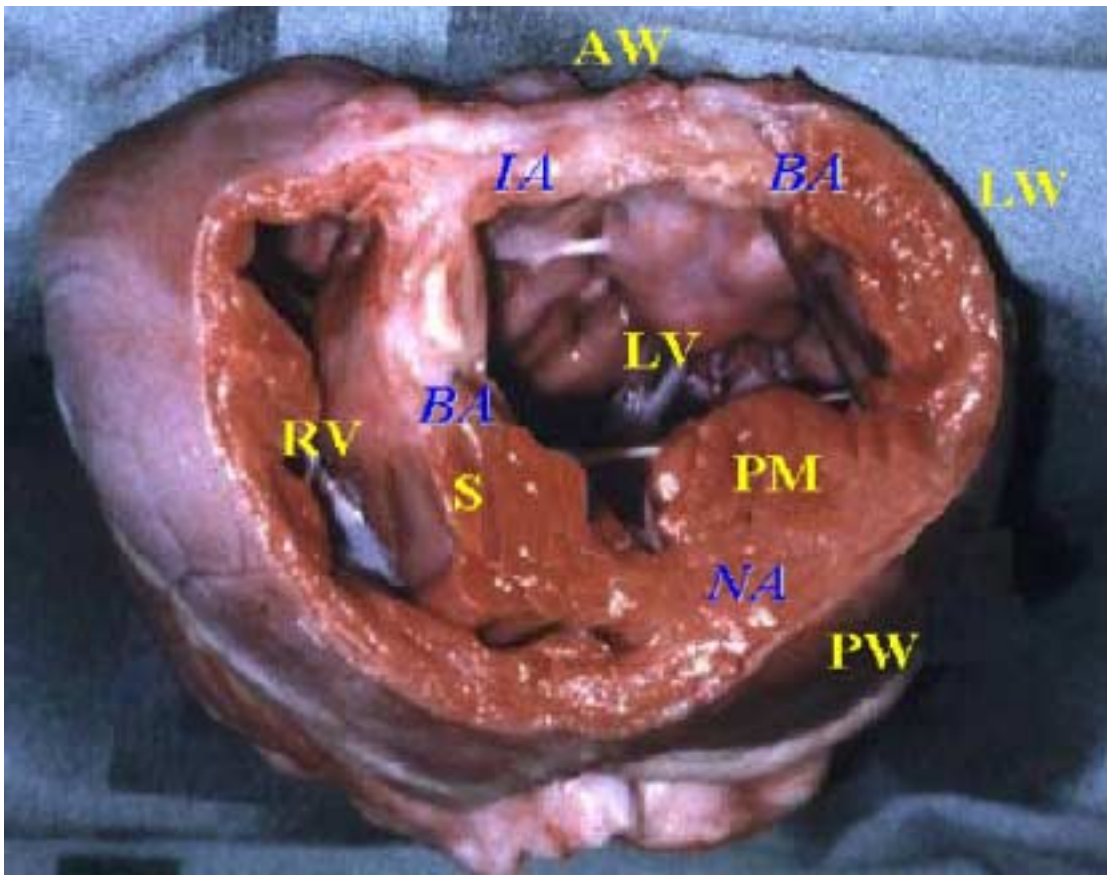

Figure 8. Representative sample of infarcted myocardium (apical view at papillary muscle level). LV – left ventricle cavity; RV – right ventricle cavity; AW – anterior wall; LW – lateral wall; PW – posterior wall; PM – papillary muscle; S – septum; IA – infarct area; BA – border area; NA – normal area.

Histopathology and immunohistochemistry

Paraffin-embedded samples were cut by microtom (5μm thick), placed on histological slides, and the following stainings were performed:

1. Hematoxylin and eosin (H&E);
2. Masson Trichrome;
3. Immunohistochemical staining for contractile (actin) and cytoskeletal (desmin) proteins;
4. Immunohistochemical staining for blood vessels;

1.H&E staining

H&E staining was performed on all slides, representing normal, border and infarct areas of the myocardium of each animal (Fig. 9). The slides were studied and characterized in cooperation with a specialist pathologist, blinded to the experimental groups.

2. Masson Trichrome staining

Masson Trichrome staining, distinguishing viable myocardial tissue (red) and fibrotic scar tissue (green), was performed on all slides, representing normal, border and infarct areas of the myocardium of each animal. (Fig.10). The staining was used to verify the validity of the visual definition of the above-mentioned areas. After the verification, representative myocardial areas were analyzed for different characteristics.

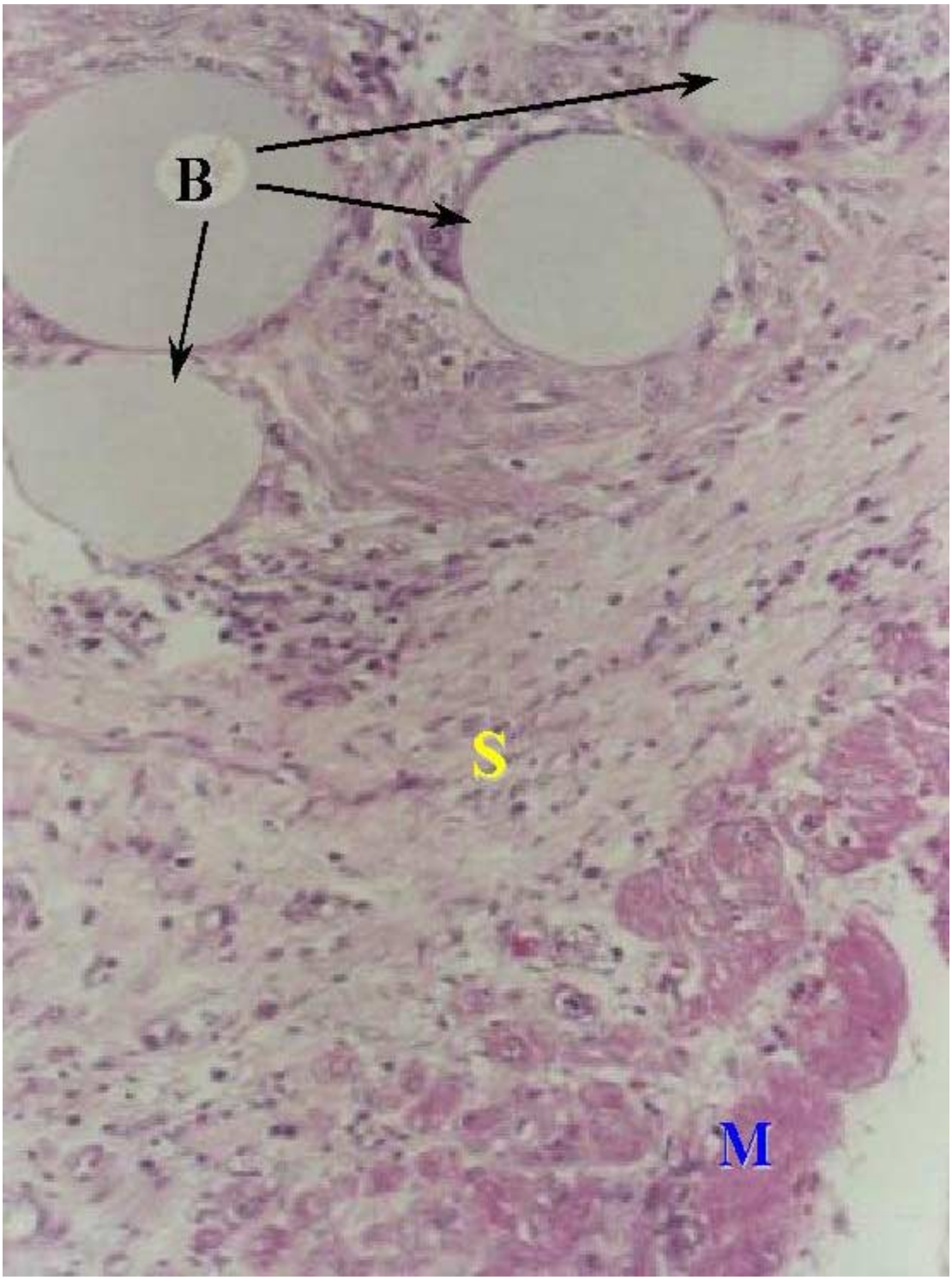

Figure 9. A. Representative sample of infarct myocardial area stained with H&E. B – affigel blue beads embolizing blood vessels; M – myocardial muscle; S – scar tissue.

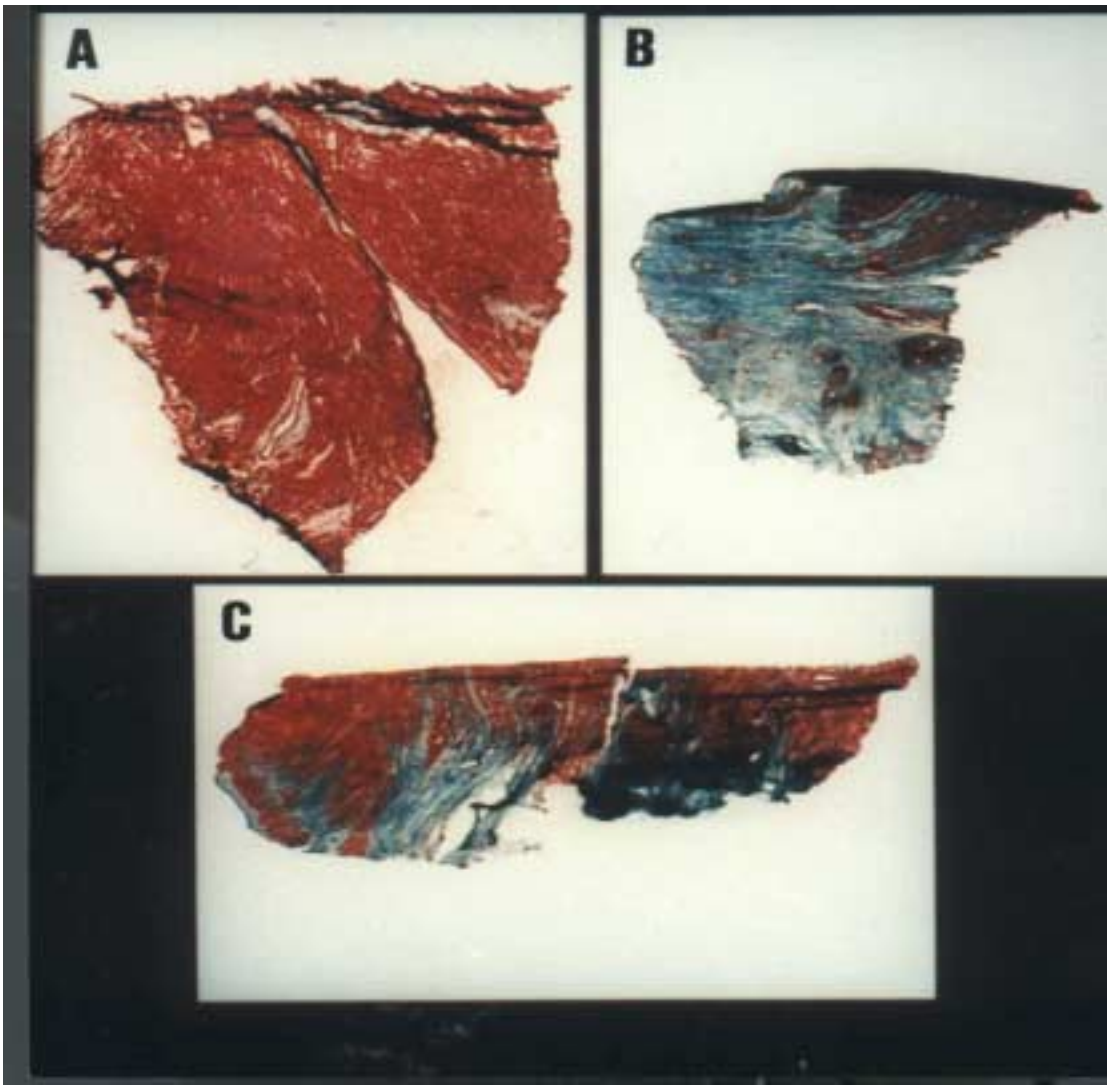

Figure 10. Representative samples of normal (A), infarct (B) and border (C) myocardial areas. The slices are stained with Masson Trichrome, distinguishing viable myocardial tissue (red) and fibrotic scar tissue (green).

3.Actin and Desmin staining

In order to evaluate the effect of IGF-I and II administration on myocardial contractile and cytoskeletal proteins, actin and desmin as representative proteins of each protein group, respectively, were analyzed in the present study. The slides from each area, of each animal, were stained with *Monoclonal Mouse IgG2a anti-actin* and *Monoclonal Mouse IgG_1 kappa anti-desmin,* respectively, using Histostatin-SP Zymed kit (51,52). The antibody to muscle specific actin reacts with a 42 kD protein specific for α actin present in skeletal, cardiac and smooth muscle and γ actin present in smooth muscle. In myocardial tissue, sarcomeric and smooth muscle actin (which may be found in blood vessel wall and in myofibroblasts) are stained. The actin and desmin containing area stained reddish brown, while the rest of the myocardium stained light-violet, scar tissue stained gray, and cellular nuclei stained blue. The quantitative evaluation of actin and desmin stained areas was performed under magnification of ×150, on all slides, from all animal. The slides were projected on a PC monitor, using Galai Scannaray/ Supercue-3-Computerized Colored Image Analyzer (Galai, Migdal Haemek, Israel). Nine fields of 23.55×10^3 μm^2 were randomly chosen for evaluation. The threshold for actin and desmin staining color was computerized and used to count the protein containing area of each field automatically. The values of actin and desmin were expressed as percentage of the total area at each field and presented as mean of 9 fields ± SD.

4. vWF staining

In order to evaluate the effect of IGF-I and II administration on the number of blood vessels, immunohistochemical staining for endothelial cells was used (41,53). The slides from each myocardial area of each animal were stained with anti-vWF antibody (1:200), using Histostatin-SP Zymed kit. Endothelial cells stained red, myocardial muscle stained light violet, scar tissue stained gray and cellular nuclei stained blue.

Nine fields on each slide representing each myocardial area were randomly chosen for stained blood vessels counting. Counting was performed using a grid inserted into the ocular (1 cm^2 divided into 10×10 squares of 1 mm^2 at ×400 magnification). All stained vessels with lumen dissected by the vertical grid lines were counted (41,53). Vascular counts for each slide were calculated as sum of counts in nine fields. The number of blood vessels per area in each group was presented as mean ± SD. Blood vessel densities in different myocardial areas were compared among the three groups of the study and this comparison served as a marker of angiogenesis in treated and non-treated animals.

Biochemistry

Frozen myocardial samples were used to extract DNA in order to assess DNA fragmentation using DNA laddering technique. DNA laddering was used as a marker of cellular death regardless of its cause (i.e. necrosis or apoptosis).

DNA laddering

To identify and quantify DNA fragmentation associated with myocytes death, ligation-mediated PCR of blunt DNA ends was used as previously described (54).

For densitometric analysis, 20 μl of PCR products for each lane were loaded on 1.5% agarose gels, followed by electrophoresis and staining with ethidium bromide (Pharmacia AB, Sweden). The bands were photographed and then scanned with a DuoScan scanner (Agfa) connected to a computer. Only bands between 150 and 1000 bp were considered fragmented DNA and served as a marker for cell death. Integrated optical density value for each lane was calculated using a Gel-Pro Analyze software (Media Cybernetics, MA, USA). Only lanes obtained on the same gel were evaluated and compared. PCR and electrophoresis were duplicated. The signal of the control group served as the reference for each area and assigned the value of 100%.

Data analyses

The two-way ANOVA with repeated measures were used for comparison between IGF-I, IGF-II and control animals. Student's t-test: two-sample assuming unequal variances was used to compare between IGF-I and II groups. The results are presented as mean ± SD (mean ± SE for DNA laddering), and a $p \leq 0.05$ was considered statistically significant.

RESULTS

Mortality

Five animals died shortly after the microembolization as the result of AMI. During the four weeks follow up period 3 more animals died (one pig in each experimental group). Finally, the number of pigs that reached the end point of the work and were subjected for euthanasia was eighteen (six pigs in each experimental group). Thus, the operative mortality in this study comprised 19%, while the total mortality was 31%.

Body weight

There was no difference in the initial body weight of all pigs (24.9±2.2, 27.0±2.4 and 27.0±2.8 kg, p=0.27, for IGF-I, IGF-II and control, respectively). Four weeks following induction of the MI there was not seen any statistical difference among the three study groups in final body weight (36.3±6.6, 37.0±5.8 and 36.7±4.0 kg, p=0.97, for IGF-I, IGF-II and control, respectively).

Blood pressure

Mean blood pressures of treated and non-treated animals before and after adenosine administration 4 weeks post-MI were compared and summarized in Table 2. No significant differences were seen in mean blood pressures among the three groups of animals 4 weeks post-MI before adenosine administration. Adenosine infusion caused a similar reduction of mean blood pressure in all animals. There was no difference in the reduced mean blood pressure among the three groups, following adenosine infusion.

Table 2: Blood pressure (mean ± SD) in IGF-I, IGF-II and control groups before and after adenosine administration, 4 weeks post infarction.

Group	**Mean Blood Pressure, mmHg**		
	Before adenosine	**After adenosine**	**Reduction (%)**
IGF-I	85 ± 11	64 ± 9	30 ± 3.1
IGF-II	84 ± 9	64 ± 9	32 ± 6.2
Control	78 ± 13	56 ± 11	30 ± 2.8
p	0.447	0.277	0.792

Heart rate

Heart rate of all animals was followed up during the study and the data were summarized in Table 3. The heart rate was similar in all animals at baseline. The MI caused an increase in heart rate of all animals, with no difference among the groups. There were no observed differences in initial heart rate among the three groups, 2 weeks post-MI and 4 weeks post-MI.

Table 3: Heart rate (mean ± SD) of IGF-I, IGF-II and control groups at different time points.

Group	Heart Rate, beats/min				
	Base	**post-MI**	**2w post-MI**	**4w post-MI**	**P**
IGF-I	107 ± 12	115 ± 14	98 ± 13	103 ± 19	0.281
IGF-II	117 ± 15	120 ± 21	117 ± 16	118 ± 13	0.983
Control	112 ± 18	116 ± 10	108 ± 8	105 ± 15	0.558
p	0.544	0.840	0.078	0.232	

Myocardial function

Myocardial function of all animals was normal at all levels before the induction of MI. No regional dysfunction following MI was observed at the basal parts of the heart. Therefore, these myocardial segments received the score of 0, and are not reported in detail. The microembolization infarction caused morphological changes in the anterior wall and septal segments (Fig. 8). It was associated with a significant myocardial functional defect in the anterior wall and septal segments at the mid-papillary muscle and apical levels within the LV of all animals. Myocardial wall motion of the anterior wall and septal segments at the mid-papillary muscle level was damaged immediately post-MI and decreased 2 hours post-MI in all groups. Improvement in myocardial wall motion of both segments at the mid-papillary muscle level was observed in IGF-I and II treated animals 2 and 4 weeks post-MI. Myocardial wall motion abnormality of the anterior wall and septal segments during the four weeks of the follow-up period was scored as an average of the damaged segments at the mid-papillary muscle level (Fig. 11). Segmental myocardial wall motion of IGF-I and II treated animals at rest significantly improved at the mid-papillary muscle level during the 4 weeks post-MI follow-up (0.3±0.4 vs. 0.6±0.7 vs. 1.2±0.6, p=0.01, for IGF-I, II and control, respectively) (Fig. 11).

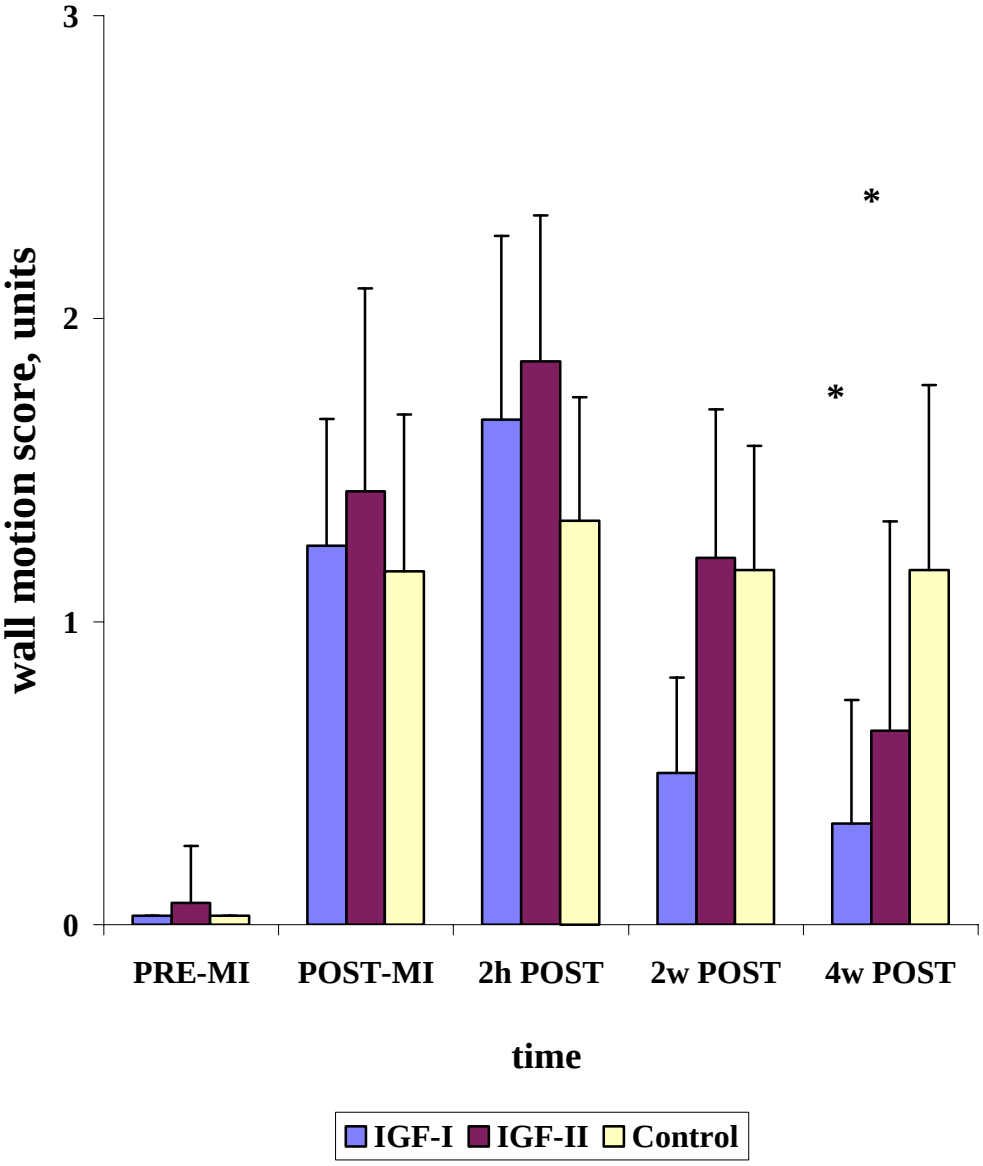

Figure 11. Comparison of myocardial wall motion abnormality in IGF-I, IGF-II, and control groups during the 4-week follow-up period at mid-papillary muscle level; pre-MI - baseline, post-MI - immediately after infarction, 2h post - 2 hours post-MI, 2w post - 2 weeks post-MI, 4w post - 4 weeks post-MI. *Indicates a significant difference from control group, p = 0.01.

The alterations in myocardial wall motion at the apical level were similar to those seen at the mid-papillary muscle level during the follow-up period (Fig 12). Myocardial wall motion abnormality of the anterior wall and septal segments during the four weeks of the follow-up period was scored as an average of the damaged segments at the apical level (Fig 12). Segmental myocardial wall motion of IGF-I and II treated animals at rest significantly improved at the apical level during the 4 weeks post-MI follow-up (0.3±0.3 vs. 0.9±0.9 vs. 1.4±0.6, p=0.02 for IGF-I, IGF-II and control, respectively) (Fig.12).

Thus, as described above, 4 weeks post-MI the segmental myocardial wall motion of IGF-I and IGF-II groups was significantly better than this of control group, with no statistical difference between IGF-I and II groups.

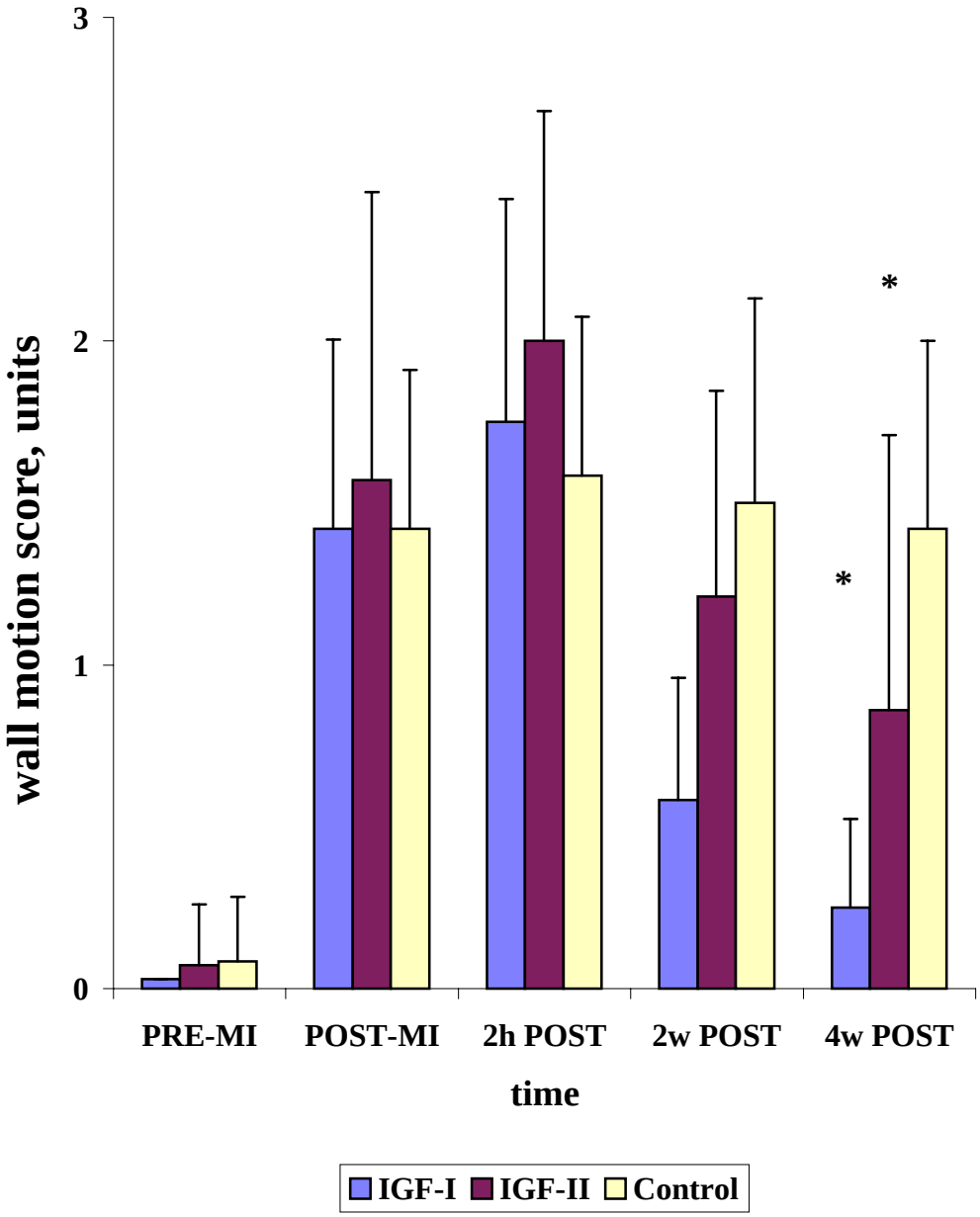

Figure 12. Comparison of myocardial wall motion abnormality in IGF-I, IGF-II, and control groups during the 4-week follow-up period at apical level; pre-MI - baseline, post-MI - immediately after infarction, 2h post - 2 hours post-MI, 2w post - 2 weeks post-MI, 4w post - 4 weeks post-MI. *Indicates a significant difference from control group, p = 0.01.

The improvement in resting myocardial function caused by IGF-I and II administration following MI occurred during the 4 weeks of follow-up period. To investigate whether myocardial functional reserve was affected during this time period dobutamine stress echocardiography (DSE) was performed 4 weeks post-MI. In order to assess myocardial functional reserve dobutamine administration with increasing doses (1 - 8 μg/kg/min) was performed. Myocardial wall motion following dobutamine administration was scored as an average of the anterior wall and septal segments at the mid-papillary muscle and apical levels (Fig 13). During the dobutamine infusion from 4 up to 8 μg/kg/min functional reserve of both segments improved in IGF-I and II treated animals and reduced in the control group. Thus, following dobutamine dose of 8 μg/kg/min the segmental myocardial wall motion of IGF-I and IGF-II groups significantly improved, while cardiac performance in the control group was reduced at the mid-papillary muscle level (0.0±0.0 vs. 0.2±0.2 vs. 0.9±0.5, p=0.03, for IGF-I, IGF-II and control, respectively) (Fig. 13).

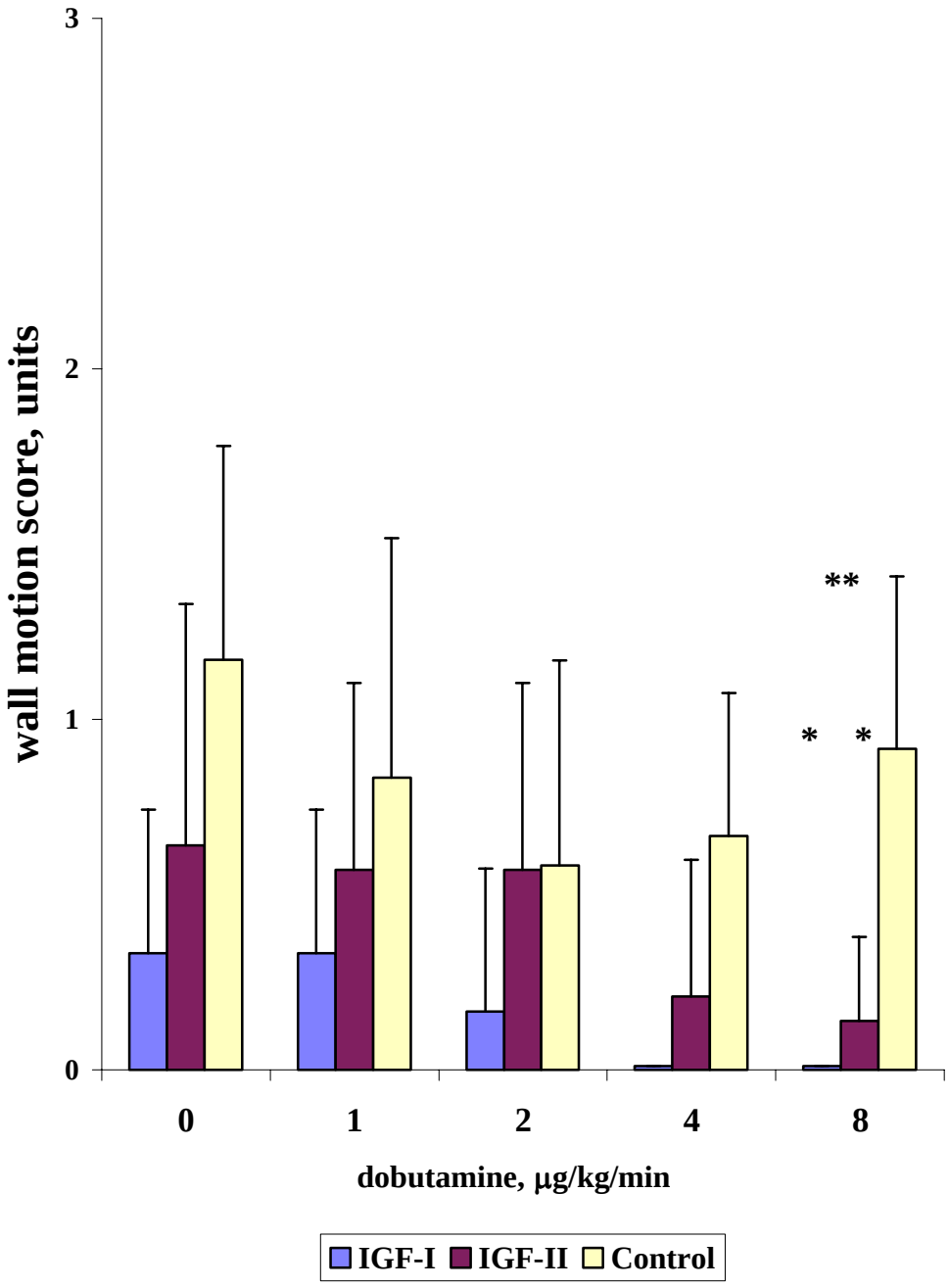

Figure 13. Comparison of myocardial wall motion abnormality in IGF-I, IGF-II, and control groups, at mid-papillary muscle level, with increasing doses of dobutamine. *Indicates a significant difference from control group, p = 0.03; **Indicates a significant difference between IGF-I and II groups, p = 0.05.

Further analyses of myocardial functional reserve following dobutamine administration were performed in the anterior wall and septal segments at the apical level (Fig. 14). After the dobutamine dose of 8 μg/kg/min myocardial function of the anterior wall segment improved in IGF-I and II treated and decreased in control animals. In the septal segment dobutamine administration up to 8 μg/kg/min improved myocardial functional reserve of the IGF treated groups. Myocardial function of the control group in the septal segment improved following 1 μg/kg/min and remained unchanged further on. Thus, following dobutamine dose of 8 μg/kg/min the segmental myocardial wall motion of IGF-I and IGF-II groups significantly improved, while cardiac performance in the control group was reduced at the apical level, (0.0±0.0 vs. 0.1±0.2 vs. 1.0±0.7, p=0.01 for IGF-I, IGF-II and control, respectively) (Fig. 14).

The improvement in myocardial functional reserve at both levels observed following dobutamine administration was also expressed in percentage, with 100% improvement in IGF-I group. Dobutamine significantly improved regional wall motion in IGF-I group as compared to IGF-II treated animals (78 ± 7%, p = 0.05), and in both IGF groups compared to control (21 ± 9%, p = 0.03) (Fig. 15).

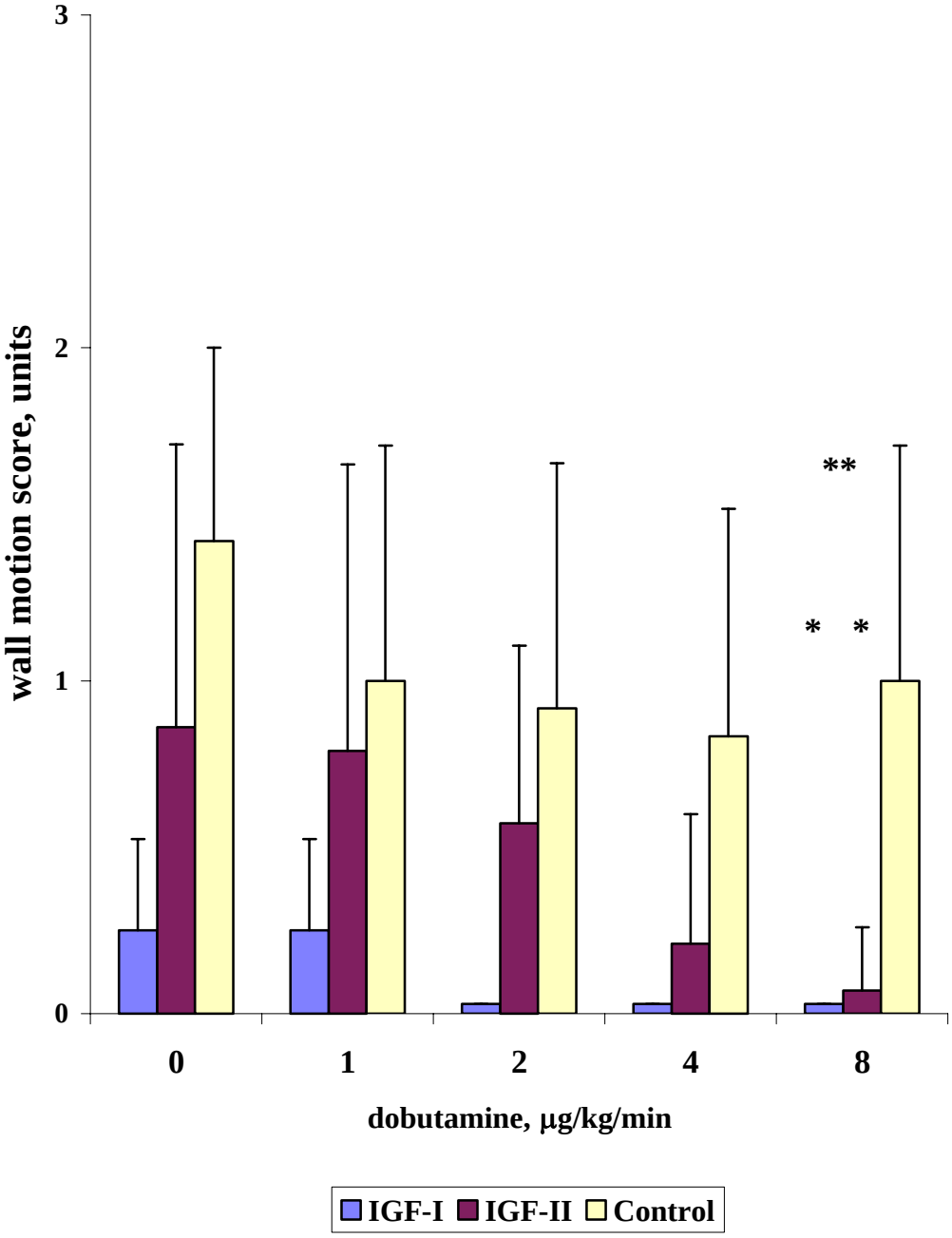

Figure 14. Comparison of myocardial wall motion abnormality in IGF-I, IGF-II, and control groups, at apical level, with increasing doses of dobutamine. *Indicates a significant difference from control group, p = 0.03; **Indicates a significant difference between IGF-I and II groups, p = 0.05.

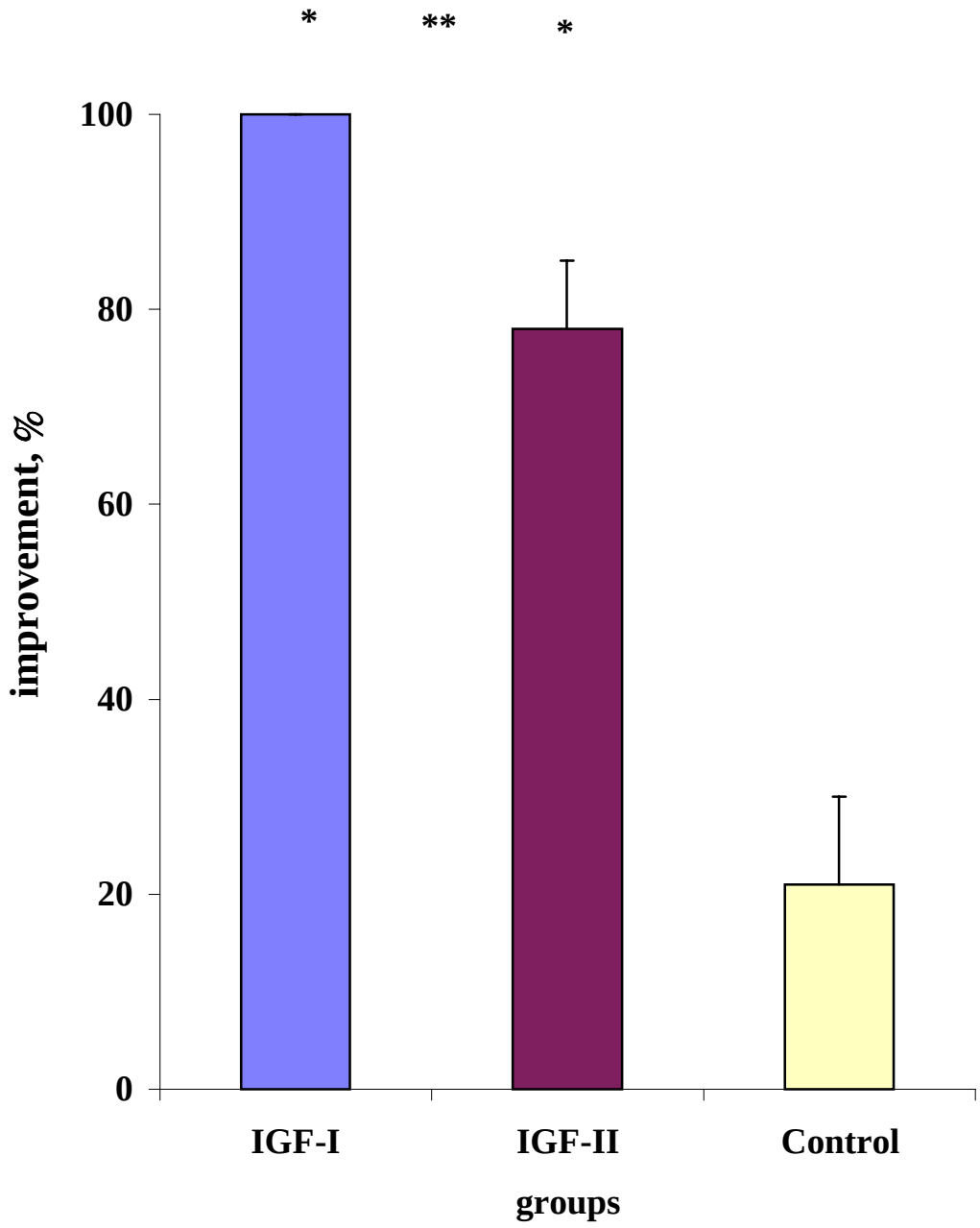

Figure 15. Comparison of improvement in myocardial wall motion abnormality following dobutamine administration in IGF-I, IGF-II, and control groups. *Indicates a significant difference from control group, p = 0.03; **Indicates a significant difference between IGF-I and II groups, p = 0.05.

To summarize the data on myocardial function observed in the present study, the location of the damaged myocardium affected by IGF treatment, the nature of MI and the relation between these two factors have to be emphasized. In this work a model of microembolization infarction was used. Microembolization causes a non-homogenous patchy type of infarction, which is different from the usual human MI caused by occlusion of a coronary artery. Thus, the damaged areas were distributed within the viable myocardium. Moreover, the microembolization in the present work was performed using beads, which contained IGFs applied to the infracted area as a treatment. Therefore, the results in myocardial function observed in the study showed an improvement in the damaged myocardial areas.

Myocardial perfusion

In order to explain the improvement in myocardial function following MI detected in IGF-I and II treated animals, myocardial perfusion analyses were carried out.

Pre-MI myocardial perfusion was within the normal range in all animals as determined by the unbiased investigator-cardiologist (Fig. 16 Pre-MI). Myocardial infarction resulted in a significant myocardial perfusion defect in the lower septal and anterior LV segments, evident in all animals (Fig. 16 post-MI). Myocardial perfusion defect was scored in the area of the damaged segments (Fig. 17A,B). No significant differences in myocardial perfusion within the septal segment, were observed among the three groups, either during the 4 week post-MI, (1.20±0.50 vs. 1.35±0.96 vs. 1.40±0.80, p=0.85) (Fig. 17A), or after adenosine administration (1.00±0.90 vs. 1.20±1.30 vs. 1.10±1.00, for IGF-I, II and control, respectively, p=0.56) (Fig. 17B). Similarly, no significant differences in myocardial perfusion within the anterior segment, were observed among the three groups, either during the 4 week post-MI, (3.50±1.00 vs. 3.25±1.00 vs. 3.35±1.04, p=0.94) (Fig. 18A), or after adenosine administration (3.10±1.16 vs. 2.75± 2.00 vs. 3.00±1.59, for IGF-I, II and control, respectively, p=0.64) (Fig. 18B).

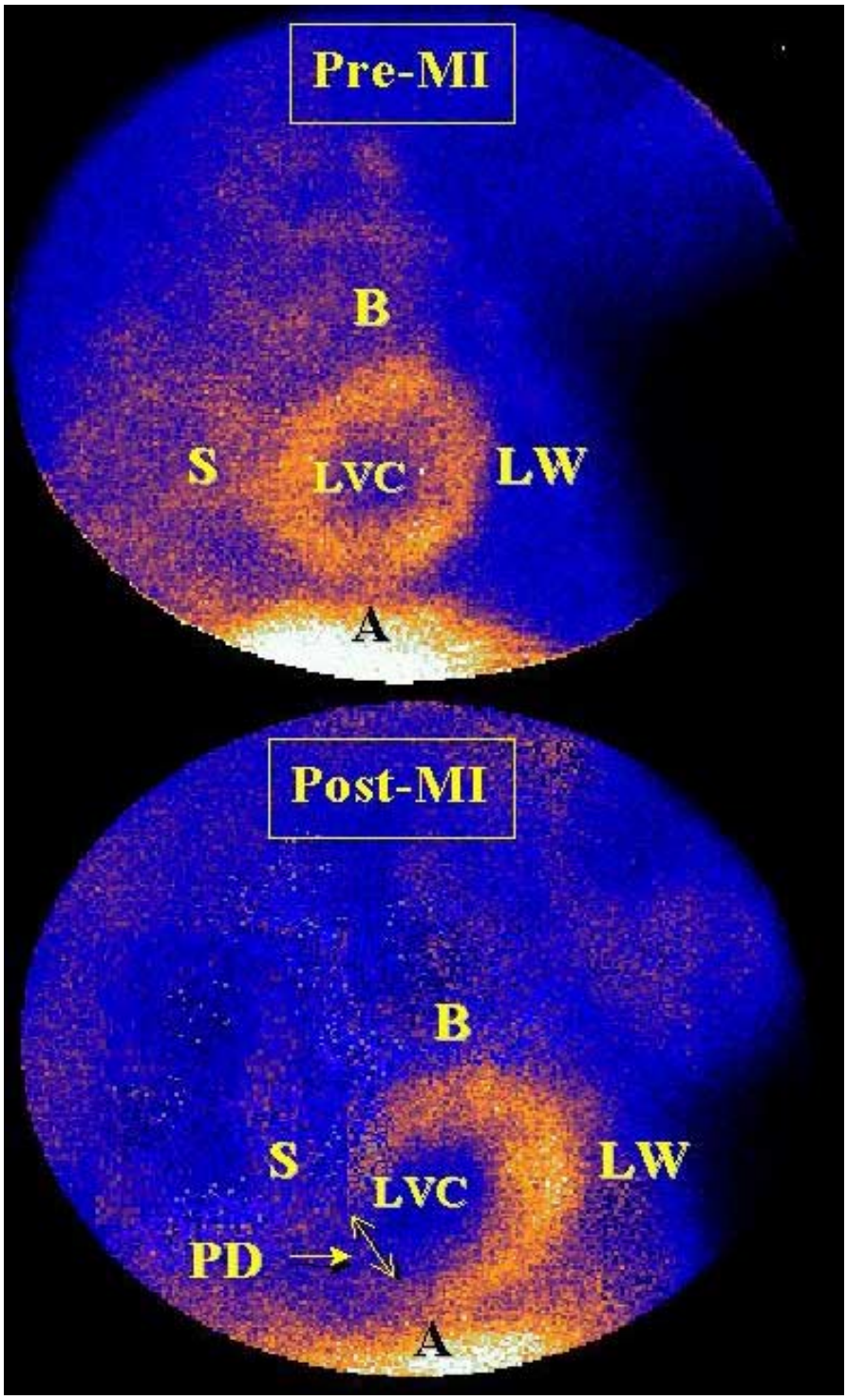

Figure 16. Representative perfusion image pre- and post-MI. LVC – left ventricle cavity; A – apex; S – septum; B – base; LW – lateral wall; PD – perfusion defect.

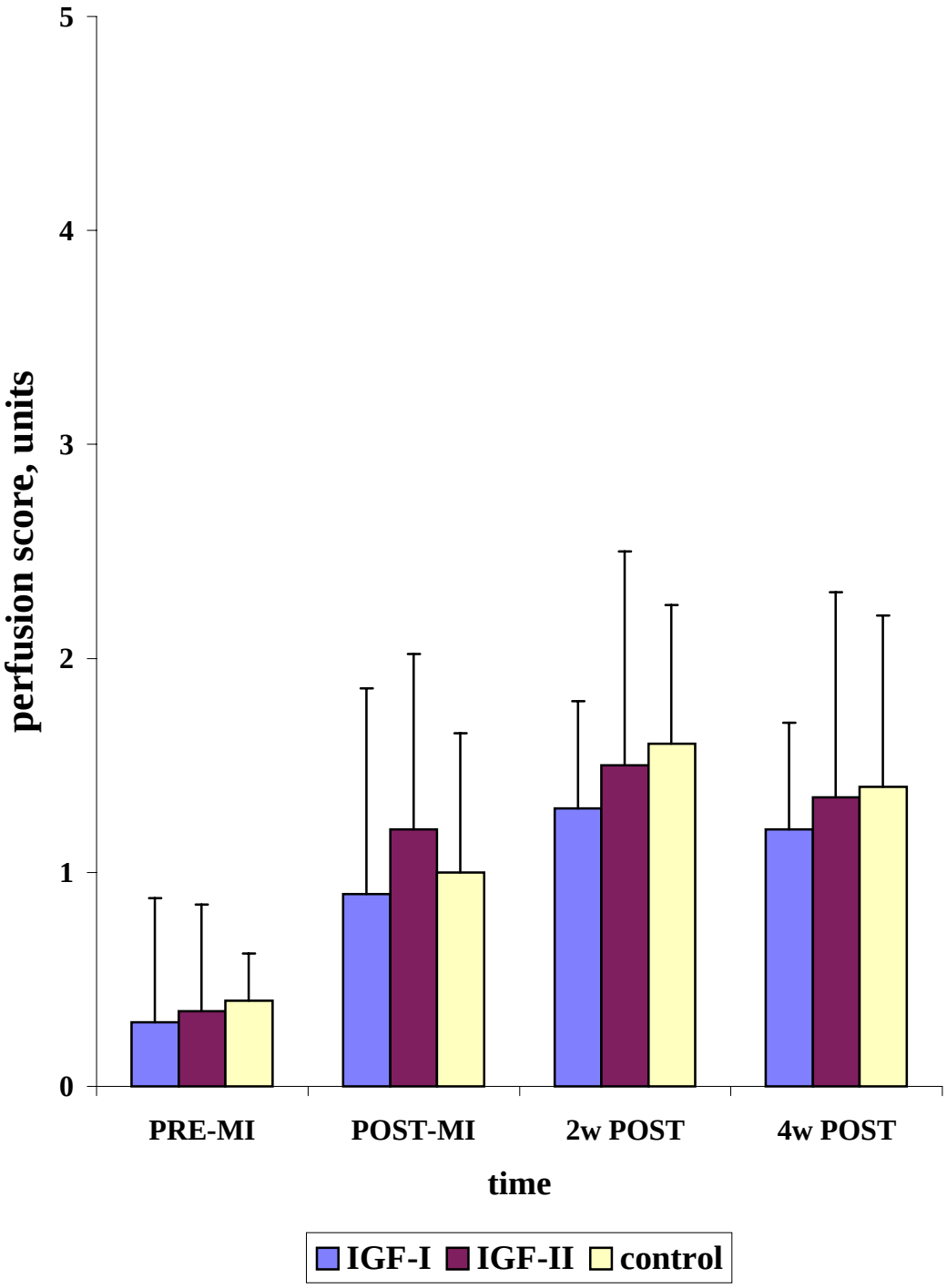

Figure 17: A. Comparison of myocardial perfusion defect in the septal segment of IGF-I, IGF-II, and control groups, during the 4-week follow-up period.

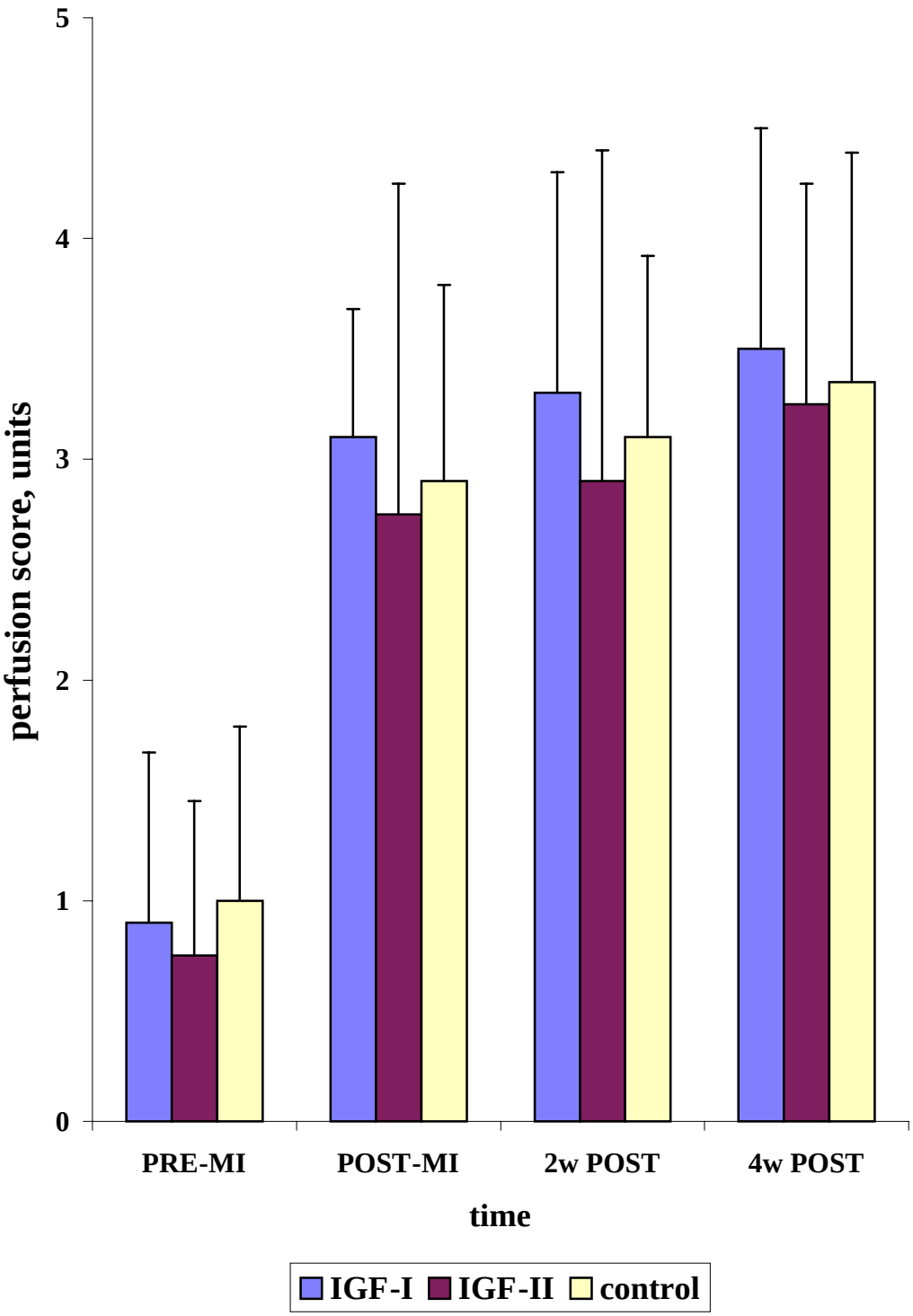

Figure 17: B. Comparison of myocardial perfusion defect in the anterior segment of IGF-I, IGF-II, and control groups, during the 4-week follow-up period.

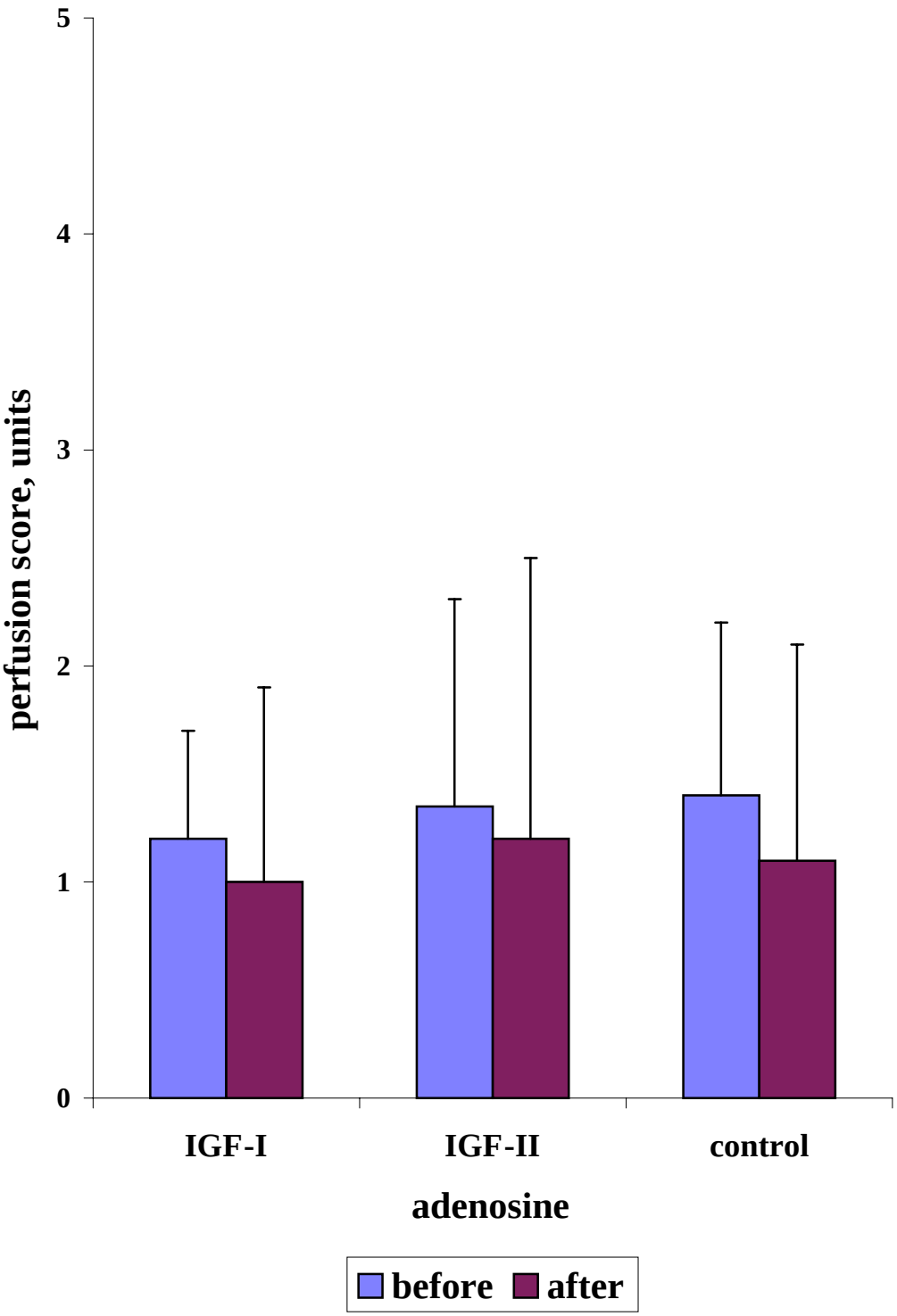

Figure 18: A. Comparison of myocardial perfusion defect in the septal segment of IGF-I, IGF-II, and control groups, 4 weeks post-MI, before and after adenosine administration.

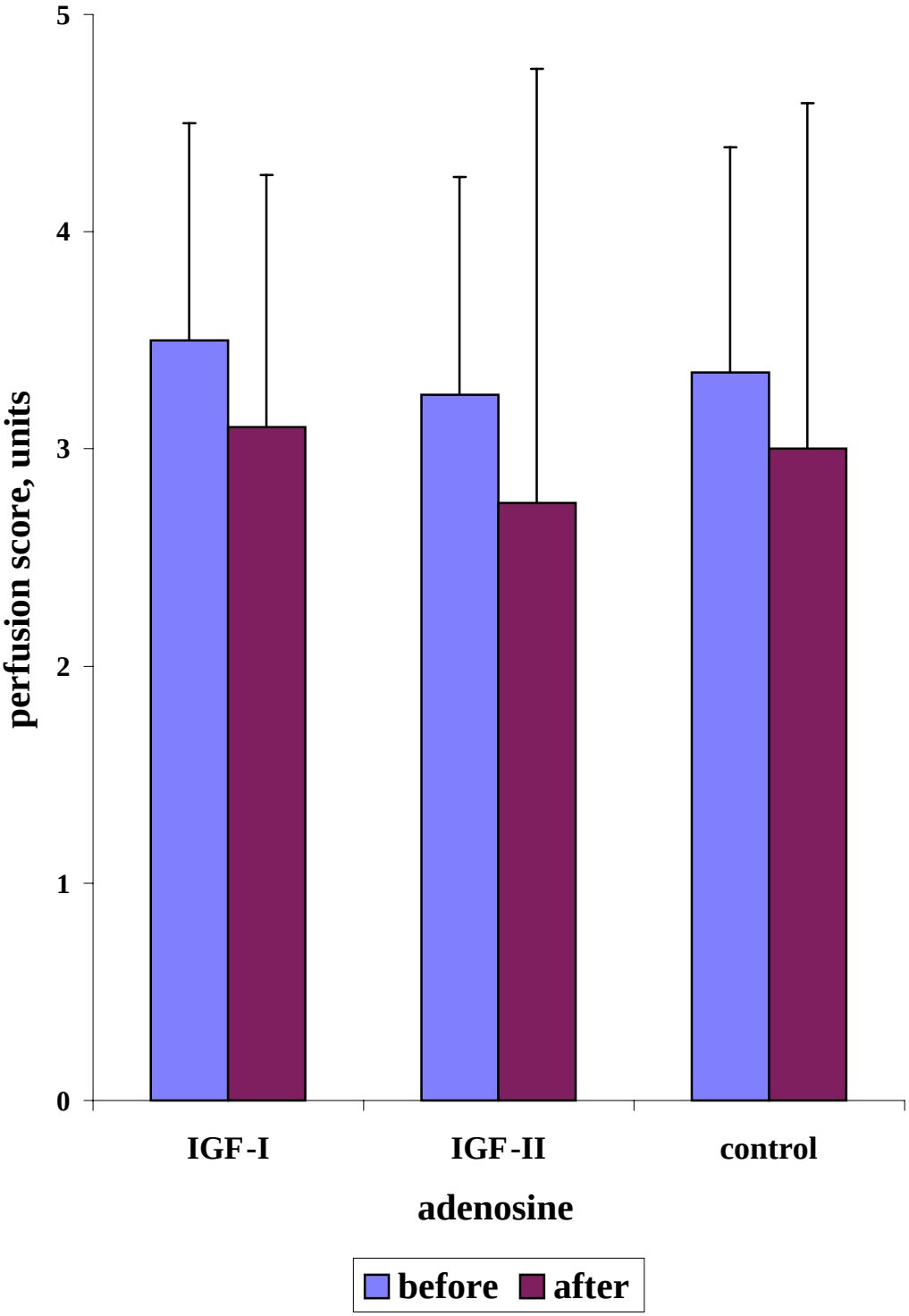

Figure 18: B. Comparison of myocardial perfusion defect in the anterior segment of IGF-I, IGF-II, and control groups, 4 weeks post-MI, before and after adenosine administration.

Serum cardiac troponin I concentration

Analyses of physiological characteristics of infarcted myocardium following IGF-I and II administration were presented in the previous sections. Serum cardiac troponin I concentration was evaluated as serum marker for myocardial damage induced by acute MI.

Serum cardiac troponin I concentration among the three groups was similar before and immediately after the infarction. However, significantly lower serum cardiac troponin I concentration was detected in IGF-I and II treated as compared to control animals 4 hours post-MI (1.90±1.23, 1.92 ±1.15 and 8.0±3.91 ng/ml, for IGF-I, IGF-II and control groups, respectively, p=0.001) (Fig. 19). Thus, IGF-I and II administration was associated with decreased serum cardiac troponin I following MI.

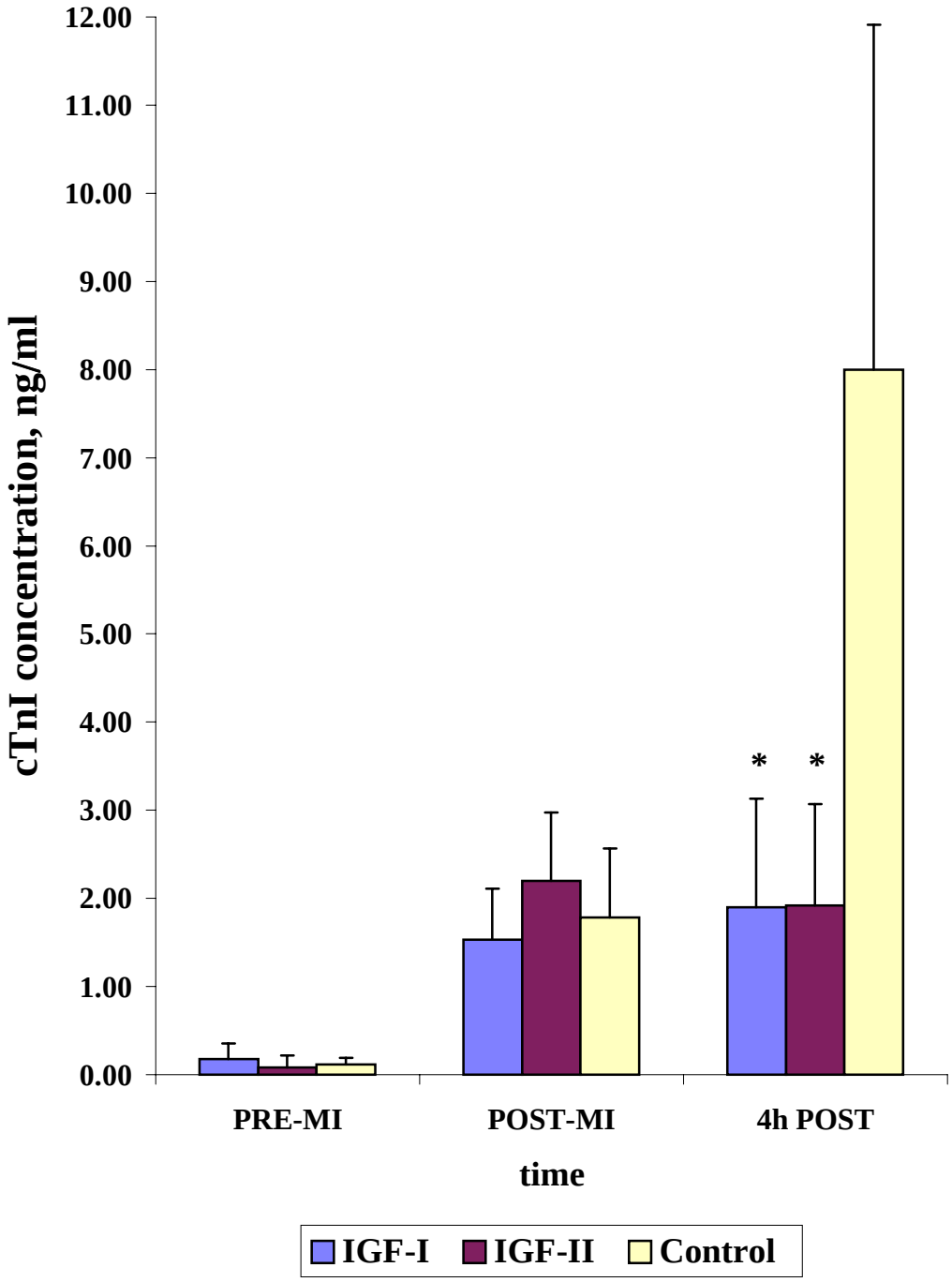

Figure 19: Serum cardiac troponin I (cTnI) concentrations in IGF-I, IGF-II, and control groups at pre-MI - baseline, post-MI – immediately post-MI and 4h post - 4 hours post-MI. *Indicates a significant difference from control, p = 0.001.

Myocardial proteins

Since IGF treatment reduced serum cTnI following MI, myocardial contractile (actin) and structural (desmin) protein contents were investigated in different myocardial areas. Therefore myocardial sample slides were stained for actin and desmin (Fig. 20A and Fig. 21A) and analyzed by computer imaging. Actin and desmin stained areas were calculated as a percent of the total muscle area at each slide (Fig. 20B and Fig. 21B).

Actin. In the normal area of the myocardium there were no differences in the actin content among the three groups. However, IGF-I and IGF-II treated animals possessed larger actin content, as compared to control group (36.7±6.4, 30.5±3.8, 25.4±4.8 %, p=0.01, and 4.5±0.2, 4.4±0.9, 2.1±0.9 %, p=0.0001, for IGF-I, IGF-II and control animals in the border and infarct areas, respectively) (Fig. 20B). Moreover, IGF-I treated group had more actin in the border area than IGF-II animals (36.7±6.4 vs. 30.5±3.8, p=0.03) (Fig. 20B).

Desmin. In the normal and border areas there was no difference in desmin content among the three groups. However, the infarct area of both IGF-I and II groups had larger desmin content than control animals (7.0±0.9, 7.1±1.0, 4.4±0.8 %, for IGF-I, IGF-II and control groups, respectively, p=0.0002) (Fig. 21B). Thus, IGF-I and II treated groups had more actin content in the border and infarct areas and more desmin content in the infarct area compared to control (Fig. 21A,B).

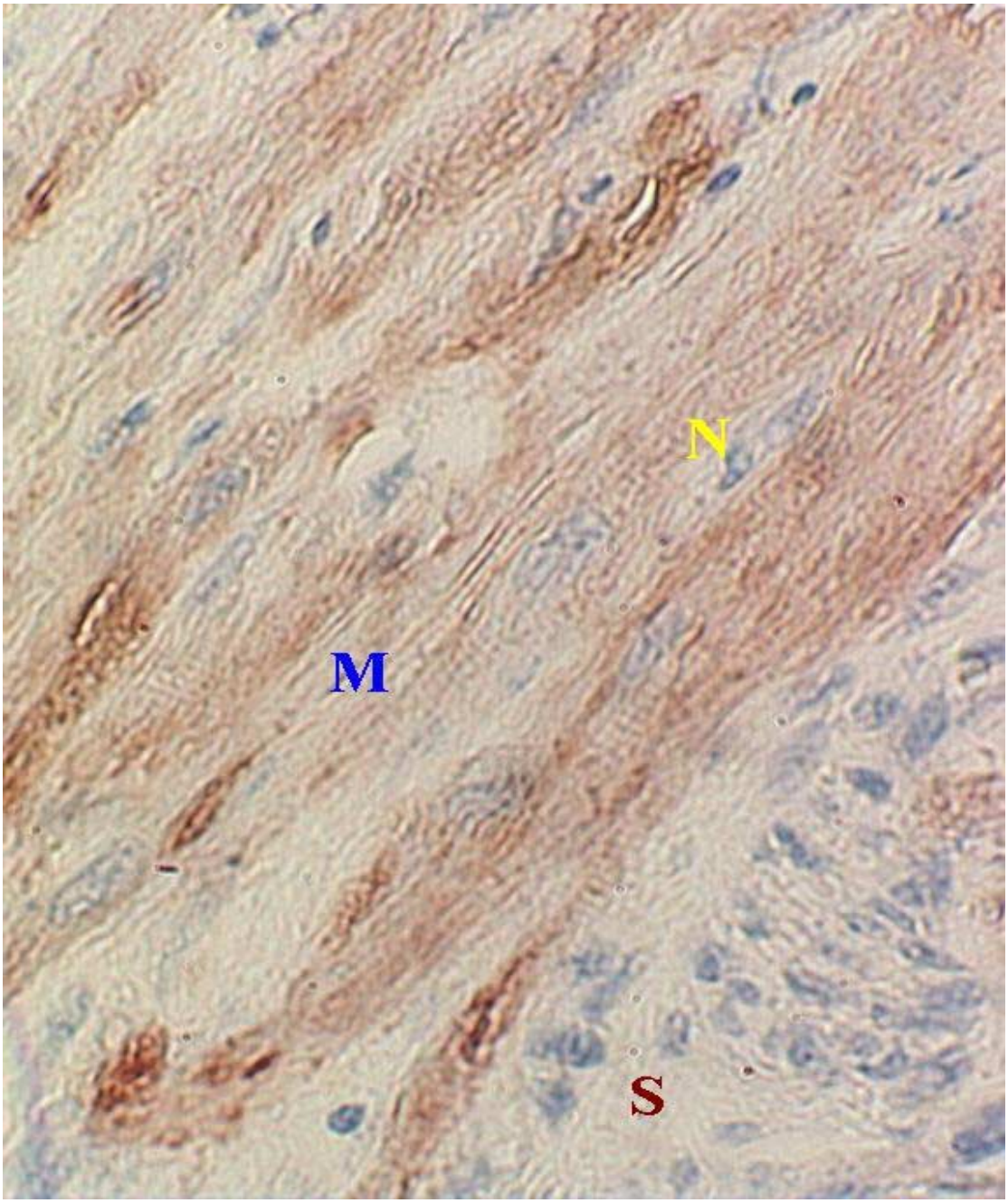

Figure 20: A. Representative sample of border myocardial area stained for actin. M – myocardial muscle remains containing actin (reddish brown stained); N – cellular nuclei (blue stained); S –scar tissue (gray stained).

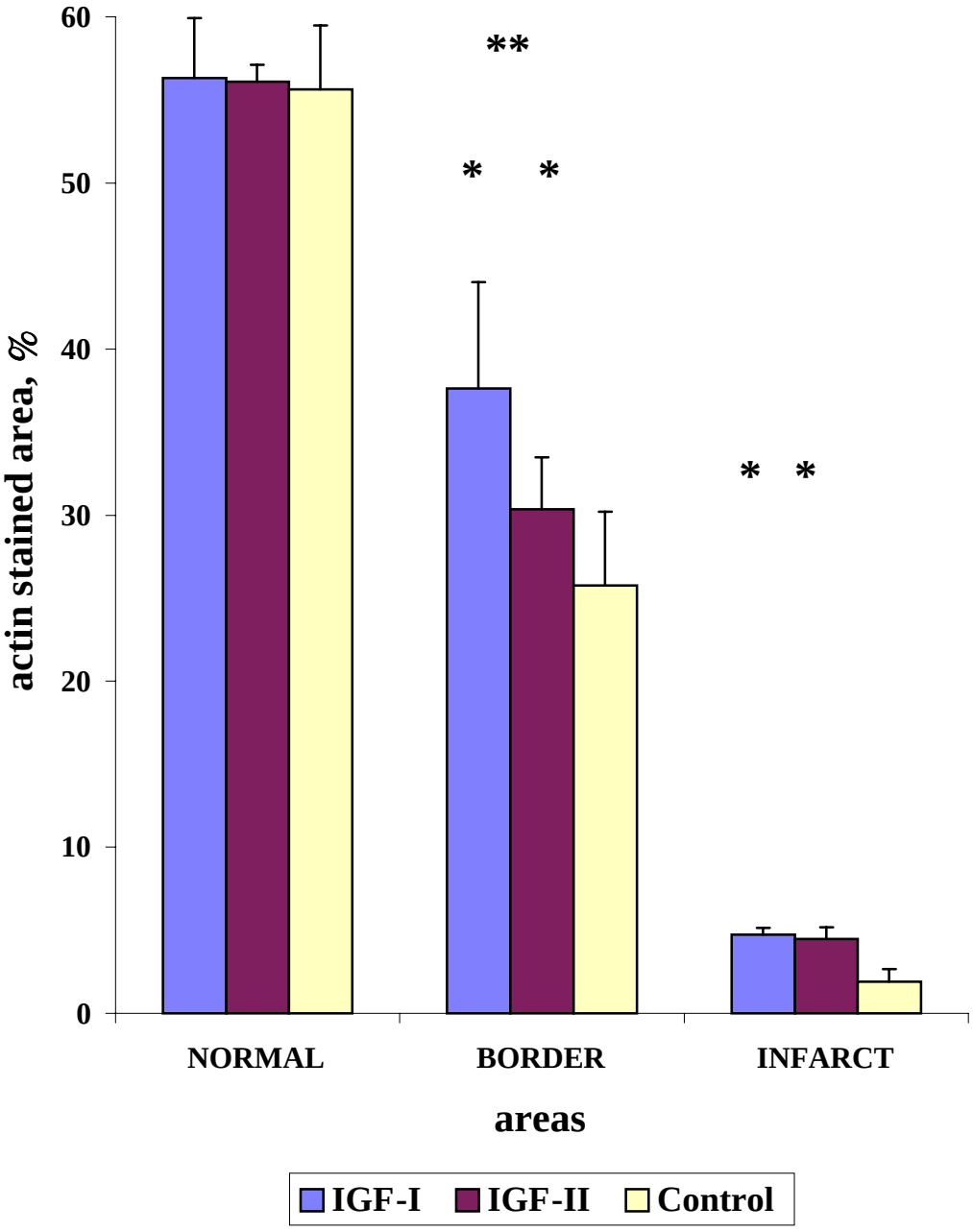

Figure 20: B. Actin content in the normal, border and infarct myocardial areas of IGF-I, IGF-II, and control groups. *Indicates a significant difference from control group in the border ($p = 0.01$) and infarct ($p = 0.0001$) areas. ** Indicates a significant difference between IGF-I and IGF-II groups in the border area, $p = 0.03$.

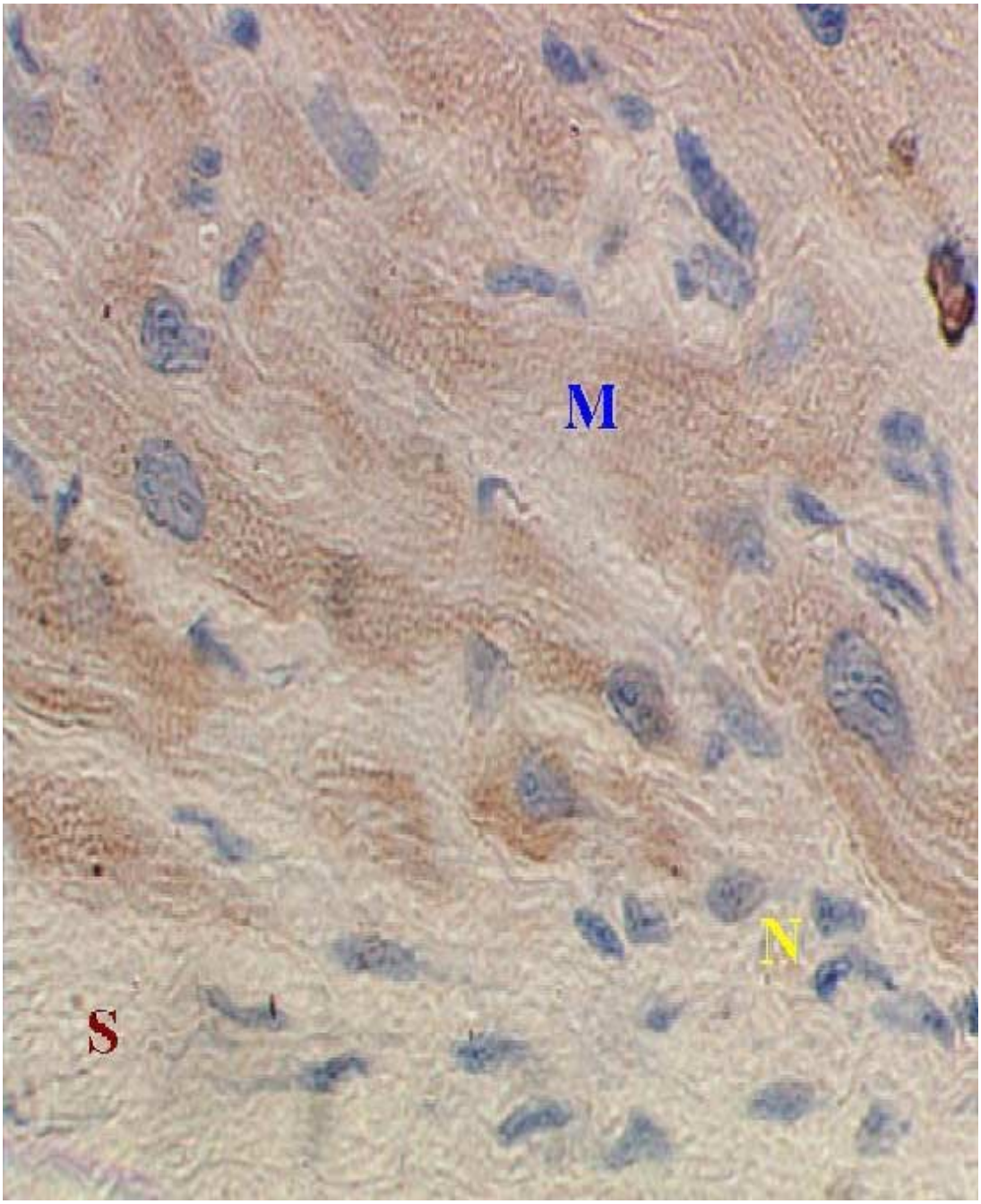

Figure 21: A. Representative sample of border myocardial area stained for desmin. M – myocardial muscle remains containing desmin (reddish brown stained); N – cellular nuclei (blue stained); S –scar tissue (gray stained).

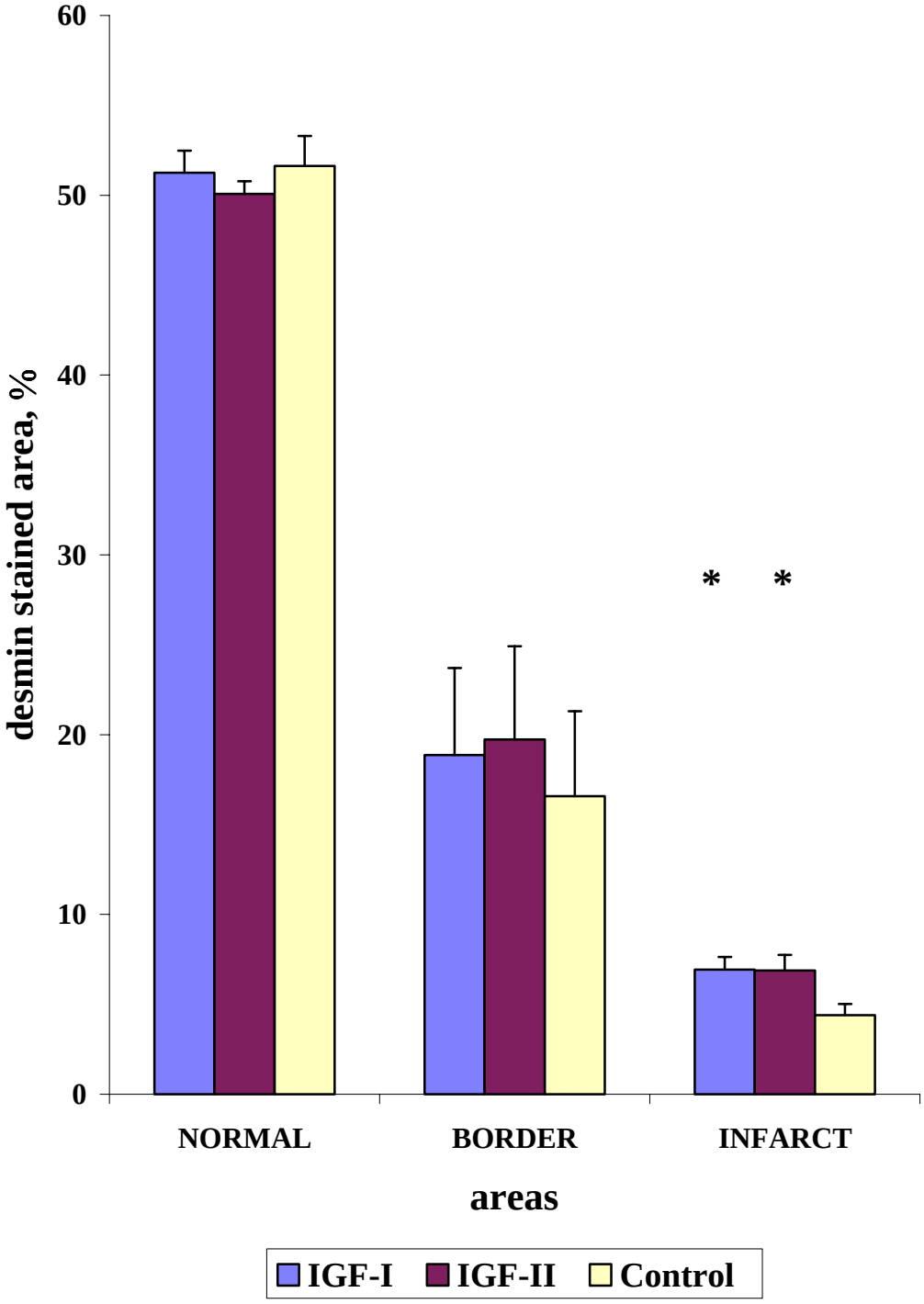

Figure 21: B. Desmin content in the normal, border and infarct myocardial areas of IGF-I, IGF-II, and control groups. *Indicates a significant difference from control group in the infarct area, p = 0.0002.

Blood vessel density

As it was shown in the previous sections, IGF administration affected myocardial function and myocardial proteins, however did not have any effect on myocardial perfusion. To investigate angiogenic effect of IGF treatment, blood vessels stained for vWF were counted in different myocardial areas. The only stained blood vessels with lumen were counted (Fig. 22A).

In the normal myocardial area there was no difference in the number of blood vessels among the three groups. However, a significantly higher number of blood vessels was detected in IGF-I, and II treated as compared to control animals (127±6, 122±9, 112±12, and 143±13, 146±9, 121±12, in the border ($p=0.04$) and infarct ($p=0.003$) areas, respectively) (Fig. 22B). Thus, more blood vessels in the border and infarct myocardial areas were seen in IGF-I and II treated animals.

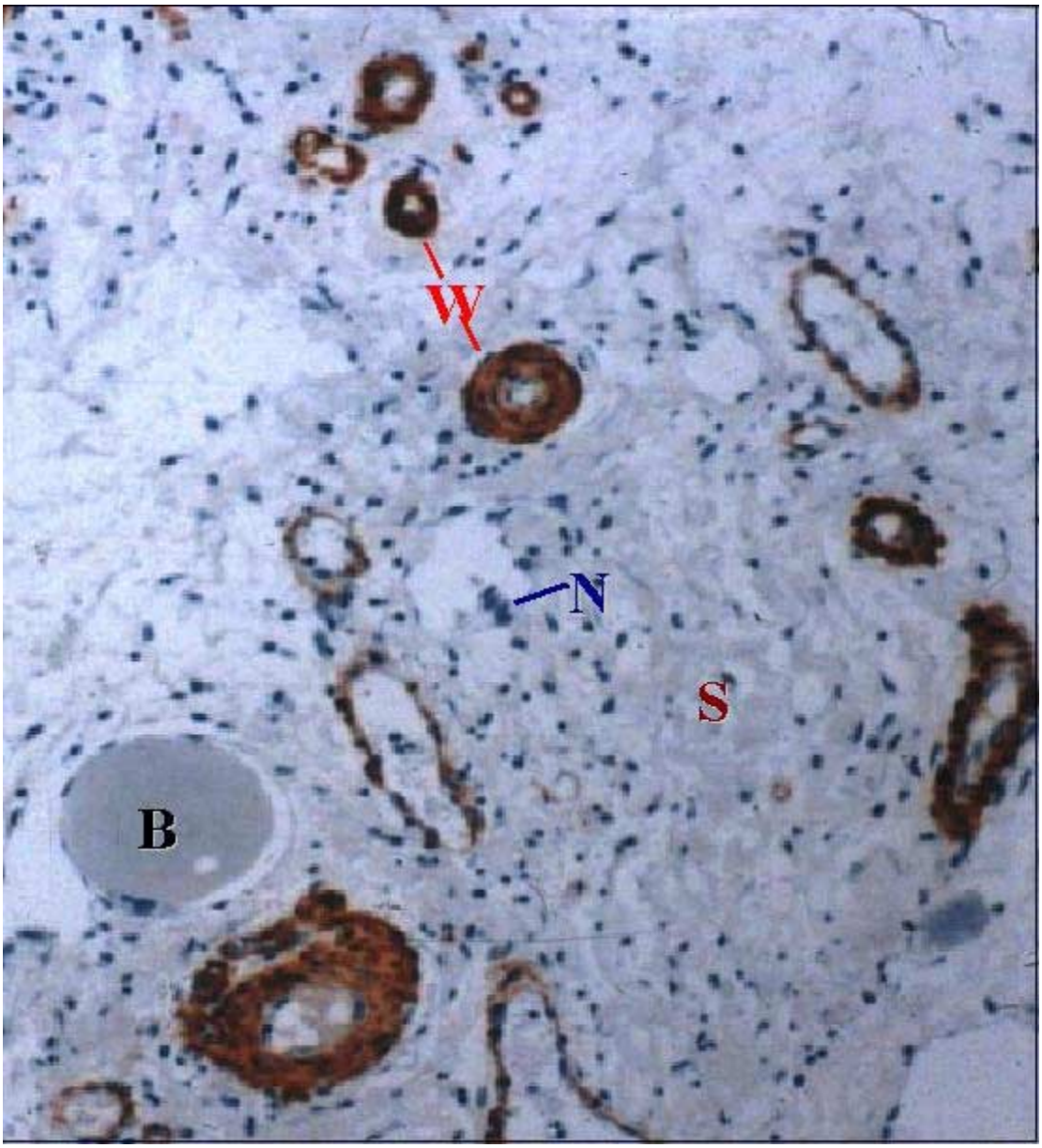

Figure 22: A. Representative sample of infarct myocardial area stained with anti-vWF. B – affigel blue bead embolizing blood vessel (dark-gray stained sphere); N – cellular nuclei (blue stained); S – scar tissue (gray stained); W – blood vessel wall (red stained).

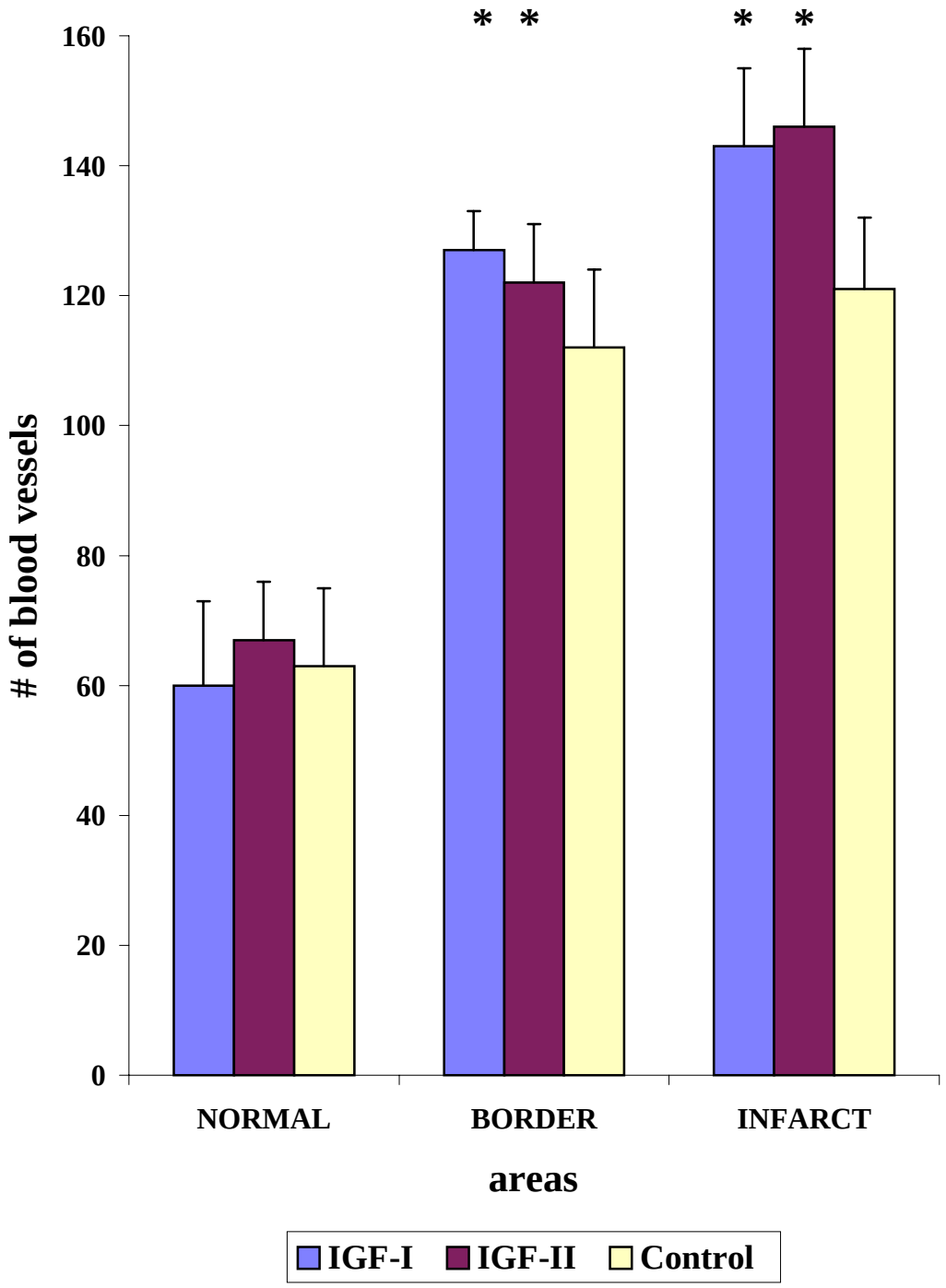

Figure 22: B. Blood vessel density in the normal, border and infarct myocardial areas of IGF-I, IGF-II, and control groups. *Indicates a significant difference from control group in the border ($p = 0.04$) and infarct ($p = 0.003$) areas.

DNA laddering

According to the data on myocardial proteins, IGF treatment appeared to affect serum cTnI concentration, and actin and desmin contents within different myocardial areas. Consequently, to make the data on myocardial proteins received by immunohistochemistry and microparticle enzyme immunoassay complete a biochemical assay was used to detect the occurrence of cell death in different myocardial areas. DNA laddering analyses were used to identify and to assess the effect of IGF administration on cell death induced by MI in different myocardial areas. DNA molecular ladder marker was used to identify bands between 150 and 1000 bp, which served as a marker for cell death (Fig. 23A.) The results obtained on the gel were analyzed by an image analyzer and presented as % of DNA laddering (Fig. 23B). No differences were observed between IGF-I and II treated animals in all myocardial areas. There were also no differences among the three groups in the normal and border myocardial areas. However, both IGF-I (23.3±8.5 %) and IGF-II (33.3±8.3 %) demonstrated reduced DNA laddering in the infarct area as compared to control animals ($p<0.05$) (Fig. 23B). Thus, IGF treatment resulted in reduction of cell death in infarct myocardial area.

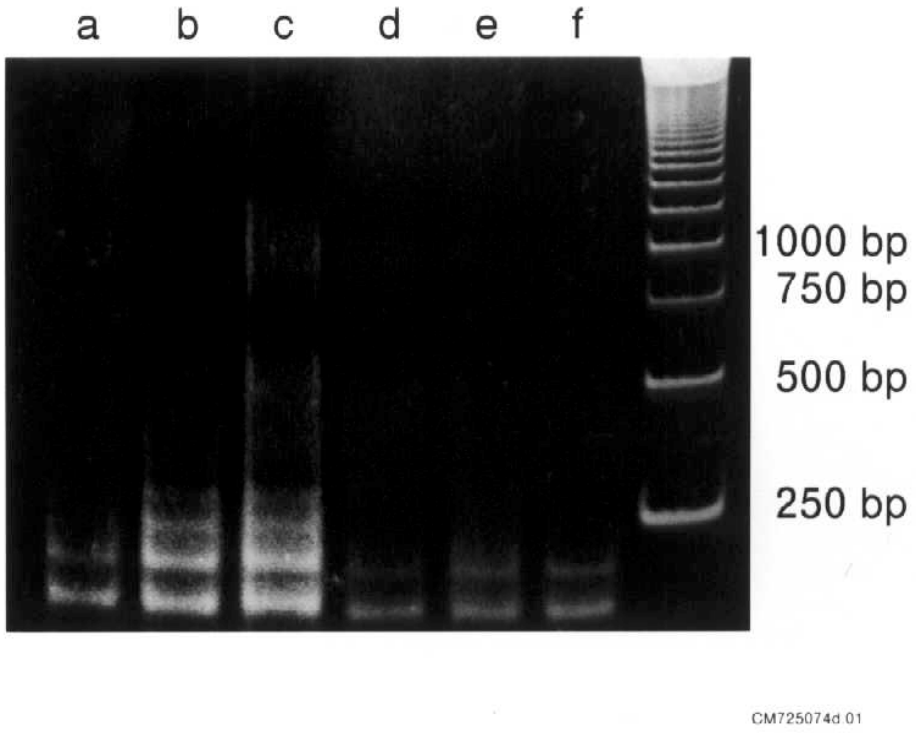

Figure 23: A. Gel electrophoresis of DNA analysis ligation-mediated PCR products. Lanes a, b, and c are DNA strand breaks from normal, border and infarct regions, respectively, from a representative control animal. Lanes d, e and f are DNA strand breaks from normal, border and infarct regions, respectively, from a representative IGF-I treated animal. The right lane is a DNA molecular weight ladder marker of double-stranded DNA (D-5042, Sigma)

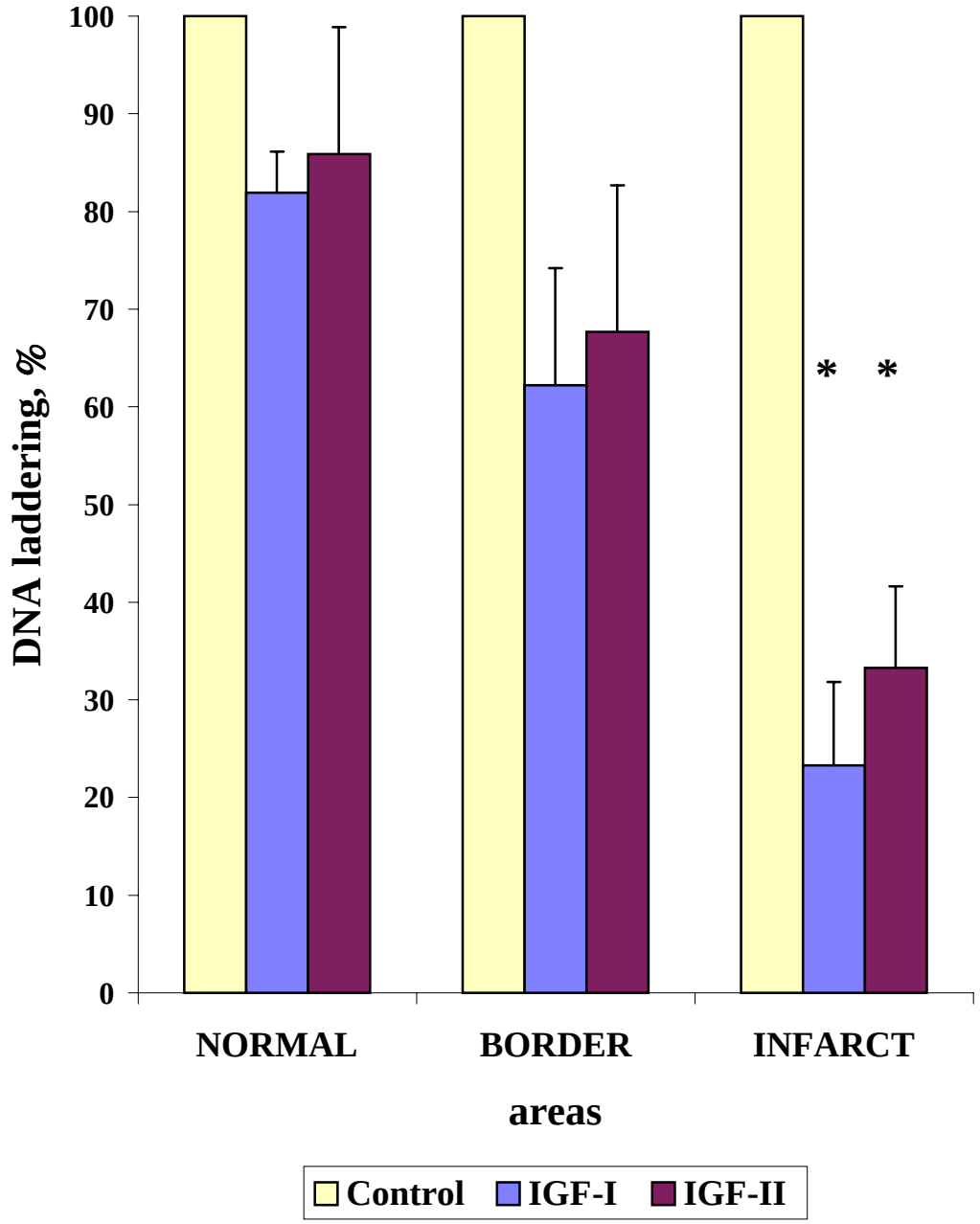

Figure 23: B. DNA laddering in the normal, border and infarct myocardial areas of IGF-I, IGF-II, and control groups (the amount of DNA laddering in the control group at each area was considered as 100%). *Indicates a significant difference from control group in the infarct area ($p < 0.05$).

DISCUSSION

The present study shows that exogenous administration of IGF-I and II improves regional myocardial function of injured regions in an experimental swine model of acute MI, with no changes in myocardial perfusion. Although a beneficial effect of IGFs on myocardial function has been reported previously (22-24,26), this is the first study comparing their effects in the same animal model of acute MI and exploring some of the underlying mechanisms of these effects.

IGF-I and II effects on myocardial function

Myocardial function during rest

As mentioned before, several works previously performed using different animal models have shown that exogenous IGF-I administration improves myocardial function following cardiomyopathy (20), ischemia and reperfusion (21), and cardiac failure (22). Moreover, involvement of total IGF-I level in the remodeling following acute myocardial infarction in human has been recently investigated (26,55). Lee et al have shown, that acute MI was associated with significant alterations in the IGF-I

system, and a higher total IGF-I level immediately after the onset of acute MI was associated with better myocardial remodeling and ventricular function (55). Accumulated evidence has indicated that IGFs play a specific role in the intricate cascade of events of cardiovascular function, in addition to their well established growth-promoting and metabolic effects (26). Following known effects of endogenous IGF on myocardial function, the effects of exogenous IGF-I and II administration were consequently investigated (26). In the work performed by Bisi and colleagues a subcutaneous administration of a low dose of recombinant human IGF-I significantly increased basal left ventricular ejection fraction of healthy human adults (56). Intravenous administration of recombinant human IGF-I improved cardiac performance in patients with chronic heart failure by afterload reduction and possibly by positive inotropism (57). In these studies IGF-I administration did not cause any hemodynamic changes (56,57). However, none of the previous studies has investigated the effect of exogenous IGF-I administration on myocardial function following MI. In our work intracoronary administered and slowly released IGF-I and IGF-II (150 μg/pig) improved myocardial function of infarcted pig hearts with no effect on hemodynamic parameters. Thus, several investigations,

including our own, have shown improved myocardial function in different settings of normal and pathological conditions, following IGF-I administration.

Dissimilar to IGF-I, there is very limited data on IGF-II administration and its effect on the infarcted myocardium. The only study that investigated exogenous IGF-II administration using the same animal model as in the present work, was performed in our laboratory by Battler and colleagues (23). An improved LV function at rest following IGF-II administration was discovered in this study (23). We found that IGF-II caused an increase in regional myocardial function, however it was smaller than that achieved with IGF-I. Thus, at the dosages administered at the present study (150 μg/pig of IGF-I and II) both IGFs exerted cardioprotection following AMI. However additional pharmacokinetic and pharmacodynamic studies are warranted.

All the previous data describing LV function following IGFs administration was collected at rest. None of the previous works investigating myocardial function following IGFs administration has investigated myocardial functional reserve. In the present work dobutamine infusion was used to investigate exogenous IGFs effect on myocardial functional reserve of the infarcted myocardium.

Dobutamine stress echocardiography

The present study showed that both IGFs improved the myocardial functional reserve following acute MI, though IGF-I was associated with better functional reserve than IGF-II. This may be explained by the interaction of IGFs with their receptors. It is known that both IGFs activate IGF-I and IGF-II receptors with relatively higher affinity to their own receptor (2). Activation of IGF-II-mannose-6-phosphate receptor, which predominantly binds IGF-II, does not induce cellular proliferation or differentiation and has no known intracellular action (2). However, activation of IGF-I receptor is known to initiate cellular growth and differentiation (2). Accordingly, a weaker effect of exogenous IGF-II than that of IGF-I may be expected.

Another plausible explanation is that IGF-I, which is naturally expressed in mature animals, interacts more effectively with adult myocytes than IGF-II, which is naturally a fetal protein (1,2), possibly due to the higher expression of IGF-I receptors in mature cells (1,2).

The dobutamine stress echocardiography was used for investigation of myocardial functional reserve, as an available and useful tool for identification of viable myocardium with reversible wall motion

abnormalities (58). Low dose dobutamine echocardiography enhances sensitivity to β–adrenergic receptor stimulation and has important clinical implications in detecting stunned and/or hibernating myocardium (59,60). In addition, inotropic response during DSE correlates with histologic evidence of hibernating myocardium (61). Moreover, dobutamine echocardiography may have a high specificity and positive predictive value for the subsequent improvement of regional LV function after revascularization (62). Thus the ability of IGFs to improve myocardial functional reserve revealed by dobutamine echocardiography may reflect myocardial protection granted by the administration of IGFs and expressed in preservation of myocardial structure and enhancement of angiogenesis following acute MI.

Preservation of myocardial structure

Serum cardiac troponin I concentration

The troponin complex consists of 3 subunits: troponin T (TnT), troponin I (TnI) and troponin C (TnC) (37). The ternary complex functions as a calcium-sensitive molecular switch that regulates the interaction between actin and myosin in skeletal and cardiac muscle (63).

Amino acid sequences of both skeletal and the cardiac isoforms of TnI exhibit approximately 40% dissimilarity in mammalian species (63). The N-terminus of human cTnI has 31 additional amino acid residues that are not present in the skeletal isoforms, allowing development of antibodies specific for cTnI (64). Unlike CK-MB, cTnI levels are undetectable in serum from patients without cardiac disease and apparently healthy individuals (37). Recently, the cardiac-specific contractile troponin I serum concentration was found to be a highly sensitive marker for the detection of myocardial-cell injury within the first few hours post acute coronary syndromes (65). Progressively higher levels of cTnI reveal increased risk of death, presumably because of the increased amount of myocardial necrosis (37).

Moreover, while both being markers for the detection of myocardial-cell injury, cTnI is a myocardial contractile protein in contrary to CK-MB which is a cytoplasmic catalytic enzyme. The cardioprotective effect of IGF administration associated with myocardial muscle preservation was shown previously in a swine model of ischemia-reperfusion (24). In addition the appearance of cTnI in the blood was shown to be proportional to its chronic loss from injured myocardium of pigs with severe left ventricular remodeling (39).

Therefore, serum cTnI concentration was measured in the present work to investigate whether IGF administration preserved contractile myocardial proteins, which may be at least partially responsible for the observed improvement in myocardial function. Several protective mechanisms initiated by IGFs may be involved in this fast prevention of infarct-induced myocardial-cell injury. In acute MI as in chronic congestive heart failure (CHF), the plasma levels of various inflammatory mediators such as interleukins and tumour necrosis factor-alpha (TNF-alpha) are rapidly elevated. TNF-alpha, produced by the inflammatory cells and the myocardium, can suppress myocardial contractility and induce the production of free radicals, which in turn can further damage the myocardium (66). Similarly, following brain injury, local increases in inflammatory cytokines trigger a reactive phenotype in astrocytes during which these cells produce their own inflammatory cytokines and neurotoxic free radicals (67). Progression of this inflammatory reaction is responsible for most neurological damage associated with brain trauma (67). Insulin-like growth factor-I (IGF-I) was found to protect neurons against a variety of brain pathologies associated with glial overproduction of proinflammatory cytokines (67). Moreover, IGF-I protected injured brain from purported

TNF-alpha-stimulated degradation (67). Furthermore, GH replacement therapy of adult growth hormone deficiency (GHD) normalized IGF-I level, which was associated with reduction of free radicals (68). According to these data IGFs may reduce the levels of pro-inflammatory cytokines and free radicals thus preserving the myocardium following acute MI. Thus, reduced serum cardiac troponin I concentrations detected in the present study suggest that administered IGFs attenuate infarct-induced myocardial-cell injury during the early post-MI hours, and this finding is directly associated with the improvement in myocardial function described in myocardial function section above.

Myocardial contractile and structural proteins

Infarct scar, a requisite to the rebuilding of necrotic myocardium following MI, is not an inert tissue as it has long been considered previously (69). Recent studies indicated that infarct scar is composed of phenotypically transformed fibroblast-like cells, termed myofibroblasts because they express alpha-smooth muscle actin and these microfilaments confer contractile behavior in response to various peptides and amines (69). These cells are nourished by a neovasculature and persist at the MI site, where they are metabolically active expressing

components requisite to angiotensin peptide generation (69). In our work more actin and desmin were detected in the infarct area of IGF-treated than in control animals. This finding conforms to possible presence of myofibroblasts within the infarct area, which may contribute to the improvement in myocardial function detected in the present study. However, further investigation has to be performed to study the influence of IGF administration on the appearance of myofibroblasts within the infarct myocardial area.

In addition, more actin was seen in the border area of IGF-treated than in control animals. As has been previously reported, autoantibodies against actin and myosin are present during and after an acute coronary syndrome (70). Moreover, they correlate with persistent troponin-I elevation at follow-up, and with late myocardial infarction (70). Serious damage in myocardial proteins caused by antibodies such as anti-actin and anti-desmin was detected in mice model of myocorditis (71). According to these data, and considering the data on cTnI concentrations and actin content observed in the present work, IGF treatment could protect myocardium, thus preventing post-MI complications including autoimmune process.

Cytoskeletal disorganization of microtubules and desmin, followed by a significant decrease of contractile myofilaments including alpha-actinin has been described in cardiac hypertrophy and CHF (72). Short-term GH supplementation improved LV pump function through increase in IGF-I plasma level in pig model of CHF as a result of favorable effects on LV remodeling and contractile processes (73). In addition, acute administration of IGF-I improved cardiac performance in patients with chronic heart failure Myocardial ischemia was also found to cause damage to myocardial contractile proteins (74). Constitutive overexpression of IGF-1 modified the detrimental effects of myocardial ischemia in mice by limiting ventricular dilation and alterations in myocardial proteins (75).

Vogt and associates have shown previously the cardioprotective effect of IGF-II administration in swine model of ischemia-reperfusion (24). IGF-II administered in their study significantly decreased myocardial infarction area as compared to the region at risk (24). The data of previous studies may support the results of the present work, suggesting involvement of cardioprotective effect of IGF administration in the observed improvement in myocardial function.

Thus, these findings may partially explain the improved myocardial function observed in the present work in IGF-treated animals. However, additional studies are necessary in order to validate these theories.

In addition, both IGFs were found to stimulate protein synthesis rate in cardiomyocytes *in vitro* (4-6), an additional mechanism, which may be responsible for our findings. Ito and colleagues have shown an increase of transcripts for skeletal α-actin but not for cardiac α-actin induced by IGF-I administered to neonatal rat cardiomyocytes (4). Similar results were observed by Adachi et al., which investigated the effect of IGF-II administration in neonatal rat cardiomyocytes (5). In another study IGF-I and II were shown to increase protein synthesis by about 70% in freshly isolated cardiac myocytes from adult rats (6). In addition, IGF-I administration was shown to stimulate cardiac hypertrophy in *in vivo* rat model of myocardial infarction (22). The results obtained in *in vitro* and *in vivo* studies clearly indicate the ability of IGFs to increase the protein synthesis rate. Thus, the combination of increased contractile and cytoskeletal proteins in the border myocardial area may reflect the hypertrophic effect of IGFs.

The histopathological analyses in the present study were performed at the myocardial structural level and were designed to investigate the mechanism of the improvement in myocardial function following IGFs treatment. Therefore, infarct, border and normal myocardial areas were analyzed for contractile and cytoskeletal proteins. However, since microembolization infarction was used in the present study, a non-homogenous patchy infarct was obtained. Thus, additional analyses should be performed in future studies to characterize the cells possessing contractile and cytoskeletal proteins within different myocardial areas. The suggested mechanism of increased by IGF protein synthesis within the border myocardial area, deserves further investigations.

DNA laddering as a marker of cellular death

According to the results of the present study, cell death occurrence was reduced in the infarct myocardial area in IGF-treated animals. Thus, IGF-I and II exogenous administration appeared to prevent cellular death following myocardial infarction. This protective effect of IGF is being recently investigated. IGFs and IGF-I receptor were shown to protect different types of cells from programmed cell death *in vitro* and *in vivo*

(30-34,76). Overexpression of IGF-I in myocytes attenuated the magnitude of accumulated damage and the extent of ongoing myocyte death in the model of coronary artery narrowing in nontransgenic and transgenic mice (75). Moreover, reduced apoptosis of cardiomyocytes associated with improved myocardial function was caused by systematically administered IGF-I in the canine model of CHF (77). Finally, IGFs were shown to protect cardiac myocytes from cell death caused by myocardial infarction (32,76,78). The results from our study show that DNA laddering within the infarcted myocardial area, which may occur as a result of cellular death, was significantly reduced by IGF-I and II administration (79). Thus, in the present study, IGFs were shown to protect cardiac myocytes from cell death caused by myocardial infarction (79). Cellular preservation coupled with contractile protein synthesis could explain the improved LV function in IGF treated animals during rest and dobutamine stress. Additional studies examining the above theories regarding IGF-I and II administration on infarcted myocardium are warranted to validate these hypotheses. In addition, DNA laddering is not a sufficient method for determination of the type of cell death. The historical development of the cell death concept was reviewed, with special attention to the origin of the terms necrosis,

coagulation necrosis autolysis, physiological cell death, programmed cell death, chromatolysis (the first name of apoptosis), karyorhexis, karyolysis, and cell suicide, of which there are three forms: by lysosomes, by free radicals, and by a genetic mechanism (apoptosis) (80). There are three documented cell death pathways known today: apoptosis, necrosis, and oncosis (81). The pathway and identification of cell injury and cell death are of key importance to the practice of diagnostic and research toxicologic pathology (82). Therefore, additional tests should be performed for specific definition of the mechanism of cell death in this model (i.e. necrosis, apoptosis, or oncosis).

Our data suggest that both IGF-I and II preserve myocardial structural and functional proteins, and prevent cell death following MI. Thus, these results may reasonably explain the improvement in myocardial function caused by IGF administration following MI.

IGF-I and II effects on myocardial perfusion and angiogenesis

Myocardial perfusion

No significant differences in myocardial perfusion were observed among the three groups during the 4-week follow-up period post-MI.

Pre-MI perfusion scores of injured segments were similar in all groups and remained so at all time points post-MI, and following adenosine administration. However, when myocardial perfusion is analyzed and evaluated by planar scintigraphic imaging, at a resolution of approximately 10mm, changes in small vessel circulation may go undetected. To examine whether IGF affects neovascularization following MI, vascular count of VWF stained vessels in different myocardial areas was used (41,53).

Myocardial angiogenesis

The data of the present study showed that exogenous IGFs increased formation of new blood vessels in the damaged and adjacent myocardial areas.

Direct and indirect actions of IGFs may be involved in their effect on angiogenesis (14-18,83-86). Kluge and colleagues found a significantly increased IGF-I mRNA expression during the period from 72 to 168h following microembolization in the pig (13). In the present study exogenous IGF-I and II were slowly released from the beads which induced the microembolization infarction. We assume that the release of IGFs occurred within 2-4 days post microembolization. Four weeks

post-MI, both IGF-I and II were found to increase the number of blood vessels in the infarct and adjacent (border) myocardial areas. The increased blood vessel density may allow for the delivery of growth factors directly to the infarcted and adjacent myocardium. Thus, the initiation of angiogenesis by exogenous administration of IGFs could have contributed to the improvement of myocardial function. However, the time course of the new vessel development could not be detected in the present work. In addition, our study did not demonstrate an IGF induced increase in blood flow. On the other hand, the “no reflow” phenomenon indicates that the presence of vessels does not always indicate the presence of flow (87). Thus, the present work can not reveal whether increased blood vessel density reduced the infarct size in the IGF-I and II treated animals. However, the initiation of angiogenesis by exogenous administration of IGFs could have contributed to the improvement of myocardial function.

The data from our study indicate, for the first time, that improved myocardial function is associated with increased angiogenesis within infarct and adjacent myocardial areas, 4 weeks after IGF-I and II administration in the pig model of acute MI.

Proposed mechanism of IGF effect

In ventricular biopsies from patients with isolated aortic stenosis and patients with aortic regurgitation IGF-I formation was positively related to velocity of circumferential fiber shortening (88). In animals, IGF-I exerted a positive inotropic effect by increasing the availability of [Ca^2+] and increased the shortening magnitude of myocytes isolated from the left ventricle of healthy adult mongrel dogs and from dogs with heart failure (89). Thus, the improvement of myocardial function caused by IGF administration in the present work may be at least partially explained by increasing the availability of [Ca^2+]. In addition, in the present work improvement of myocardial function was associated with preservation of myocardial structure in post-infarct IGF-I and II treated animals. The mechanisms of preservation of myocardial structure post-infarction may fall into three broad categories. The first is increasing myocardial oxygen supply following MI. The second is decreasing myocardial oxygen demand following MI. The third is prevention of cell death induced by MI. In the present work the improvement in myocardial function was seen 2 weeks following IGF treatment and it was associated with preserved myocardial structure and increased blood vessel density detected 4 weeks following IGF

treatment. No significant differences in myocardial perfusion, blood pressure and heart rate were observed among the three groups during the study. Thus, under the setup of the current investigation, the contribution of the increased blood vessel density to the improved myocardial function remains unknown. Consequently, there is no evidence of any increase in myocardial oxygen supply while decrease in myocardial oxygen demand was less likely to occur in the IGF-treated animals. Therefore, it is conceivable that IGF administration preserved myocardial structure and thus improved myocardial function of the treated animals by the third mechanism, namely prevention of cell death.

Differences in the effects of IGF-I and II

In the present study, IGF-I administration was more efficacious in improving myocardial functional reserve of infarcted pig than IGF-II. In addition, more actin was detected in pigs treated by IGF-I following MI, as compared to IGF-II. In the present work both factors were given in a dose of 150 μg/pig. However, it is uncertain whether this dose is optimal for both IGF-I and II. In addition, it is unknown whether the same doses of IGF-I and II are equivalently potent. Thus, to establish an explanation

for these matters, pharmacodynamics and pharmacokinetics studies of intracoronary IGF administration following MI are warranted.

Limitations

The present study examined the effects of exogenous IGFs in a patchy type of myocardial infarction. This is different from the usual human MI caused by occlusion of an epicardial coronary artery. The extrapolation of the results of the study to the "homogenous" MI should be done with caution.

Only a limited number of myocardial cytoskeletal and contractile proteins have been examined in the present study. While showing promise as possible contributors to preserved myocardial function in IGF-treated hearts, a more complete investigation should be conducted to consolidate the findings.

The expression of IGF, and their receptors or other growth factors in different myocardial areas was not investigated in the present work. However, the expression of growth factors and their receptors may significantly contribute to the understanding of the mechanisms responsible for the effects of exogenous administration of IGF observed

following MI. Therefore, an investigation of this expression is warranted in the future studies.

Conclusions

The intracoronary administration of IGF-I and II in the post-MI pig model resulted in improved myocardial function and functional reserve. This was associated with preservation of myocardial structural and contractile proteins in the infarct and border areas. These findings may form a basis for the investigation of the role of IGFs in the treatment of MI.

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אוניברסיטת תל-אביב

הפקולטה לרפואה ע"ש סאקלר

החוג לפיזיולוגיה ופרמקולוגיה

השפעת מתן גורמי הגדילה דמויי אינסולין I ו-II (IGF-I and II) על תיפקוד, פרפוזיה ומבנה שריר הלב לאחר מיקרואמבוליזציה כלילית במודל אוטם שריר לב בחזיר

חיבור לשם קבלת התואר "דוקטור לפילוסופיה"

מאת

קוטלר ארקדי-אבי

הוגש לסנאט של אוניברסיטת תל-אביב

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עבודה זו נעשתה בהנחיית

ד"ר מיקי שיינוביץ

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השפעת מתן גורמי הגדילה דמויי אינסולין **I** ו-**II** **(IGF-I and II)** על תיפקוד, פרפוזיה ומבנה שריר הלב לאחר מיקרואמבוליזציה כלילית במודל אוטם שריר לב בחזיר

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תקציר

רקע. IGF-I ו-IGF-II הם גורמי גדילה בעלי מבנה דומה לפרו-אינסולין. גורמי גדילה אלה הינם פוליפפטידים המורכבים משרשרת אחת בעלת שלושה גשרי די-סולפיד המכילה 70 (IGF-I) ו-67 (IGF-II) חומצות אמינו. מקור ה-IGF-I הוא בתאים בוגרים בעוד שמקורו של IGF-II בתאים אמבריונליים.

שני גורמי הגדילה IGF-I ו-IGF-II פועלים באופן אוטוקריני ופאראקריני וידועים כמעודדי חלוקה ושגשוג של תאי רקמות שונות כולל תאי שריר הלב. מספר עבודות הראו כי גורמים אלה משרים היפרטרופיה בתאי שריר הלב הן in vitro והן in vivo. שניהם מעודדים את סינטזת החלבונים בתאי שריר הלב של חולדה in vitro. ביטוי מוגבר של mRNA של IGF-I ו-IGF-II נראה בתאי שריר הלב בוגרים של חולדה שעברו היפרטרופיה. בנוסף, מחקרים שבדקו היפרטרופיה של שריר הלב בעקבות אוטם שריר הלב הראו כי קיימת עליה בהתבטאות האנדוגנית של IGF המלווה בעליה ברמת הרצפטורים לפקטורי גדילה אלה. מתן חיצוני של IGF-I ו-IGF-II גרם לשיפור בתפקוד הלב לאחר קרדיומיופטיה, איסכמיה ורהפרפוזיה, אי ספיקת לב ואוטם שריר הלב.

נוסף על כך, IGF-I ו-IGF-II מנעו מוות תאי מתוכנת (מת"מ) במספר סוגי רקמות כולל תאי שריר הלב. נמצא כי מוות תאי שריר הלב מתרחש בלב לאחר אוטם שריר לב חריף ו\או רהפרפוזיה כתוצאה של מת"מ וכן כתוצאה מנמק. בהתחשב ביכולת של IGF-I ו-IGF-II להגן על התאים מפני מת"מ, ניתן לצפות כי מתן חיצוני של פקטורי גדילה אלה יגן על תאי שריר הלב לאחר אוטם שריר לב חד.

באף אחד מהמחקרים הקודמים שבדקו מתן IGF לאחר אוטם שריר הלב לא נבדקה השפעת פקטורים אלה על פרפוזית שריר הלב. זאת למרות שהוכח כי IGF-I ו-IGF-II משרים הוצרות כלי דם חדשים באופן ישיר ודרך ויסות של פקטור גדילה של אנדוטל של כלי דם (VEGF) במספר סוגי רקמות. לכן, ניתן לצפות להשפעת IGF-I ו-IGF-II המגבירה את הוצרות כלי דם חדשים לאחר אוטם שריר הלב.

שיטות. עשרים ואחד חזירים ממין נקבה עברו אוטם שריר הלב חד באמצעות מיקרואמבוליזציה כלילית הגורמת לאוטם דיסטלי כמתואר בעבודות קודמות. ארבעה עשר חזירים קיבלו IGF-I ו-IGF-II (שבעה חזירים בכל קבוצה) ושבעה חזירים קיבלו אלבומין חזיר ושימשו כביקורת. דגימות דם ורידי נאספו מכל חזיר לפני, מייד ו- 4 שעות לאחר השריית האוטם במטרה למדוד ריכוז של טרופונין I ממקור שריר הלב בנסיוב. בדיקה אקוקרדיוגרפית והדמיית פרפוזית שריר הלב בוצעו במשך ארבעה שבועות של תקופת המעקב לצורך הבחנת השינויים בתפקוד ופרפוזית שריר הלב, בהתאם, לאחר מתן IGF-I ו-IGF-II. בנוסף, בכדי לבחון תפקוד ופרפוזית שריר הלב בתנאי עקה פרמקולוגית ארבעה שבועות לאחר השריית האוטם בוצעו בדיקה אקוקרדיוגרפית לאחר מתן דובוטמין והדמיית פרפוזיה לאחר הזלפת אדנוזין. ארבעה שבועות לאחר השריית האוטם החיות הוקרבו בהרדמה מלאה. הלבבות הוצאו במהירות ודגימות המייצגות את אזור האוטם, אזור הגבולי לאוטם ואזור הבריא של שריר הלב הוקפאו במהירות בחנקן נוזלי ולאחר מכן אוחסנו בטמפרטורה של 80°- מעלות צלזיוס עד לאנליזה. סדרה נוספת של דגימות שריר הלב מאותם האזורים נחתכה ועברה קיבוע ב- 4% פורמלדהיד ולאחר מכן עברה קיבוע בפרפין. הדגימות הקפואות שימשו לבדיקת

קיטוע של DNA באמצעות שיטת DNA laddering. דגימות שריר הלב שעברו קיבוע בפרפין נצבעו ל: 1) המטוקסילין-אוזין (H&E); 2) חלבון מתכווץ (אקטין) וחלבון מבנה התא (דזמין); 3)סמן לתאי אנדותל Von Willebrand Factor (vWF).

תוצאות. לפני השריית האוטם תפקוד שריר הלב של כל החיות היה תקין בכל רמות שריר הלב. מיקרואמבוליזציה כלילית גרמה לפגם בתפקוד שריר הלב בכל החיות. לא נראו בעיות בתפקוד הלב באזור הבסיס הלב. תפקוד שריר הלב באזורים הסמוכים לאוטם בחיות שטופלו ב-IGF השתפר באופן מובהק ארבעה שבועות לאחר השריית האוטם ($p=0.01$). לאחר מתן דובוטמין תפקוד שריר הלב באזור האוטם של החיות שטופלו ב-IGF-I היה טוב יותר באופן מובהק לעומת זה של החיות שטופלו ב-IGF-II ($p=0.05$), ושניהם היו טובים יותר בהשוואה לחיות הביקורת ($p=0.03$). פרפוזית שריר הלב היתה תקינה בכל החיות לפני השריית האוטם. כתוצאה של השריית האוטם פרפוזית שריר הלב נפגעה בכל החיות. פרפוזית שריר הלב הייתה דומה בכל החיות בכל נקודות הזמן של תקופת המעקב. קצב הלב ולחץ הדם היו גם הם דומים בכל החיות במשך תקופת המעקב. בחיות המטופלות ב-IGF נראה ריכוז נמוך יותר של טרופונין I ממקור שריר הלב בנסיוב ($p=0.001$) 4 שעות לאחר השריית האוטם. בנוסף, בקבוצות ה-IGF נספרו יותר כלי דם באזור הגבולי ($p=0.04$) ובאזור האוטם ($p=0.003$) לעומת קבוצת הביקורת. בחיות שטופלו נראה יותר אקטין באזור הגבולי לאוטם ($p=0.01$) ואזור האוטם ($p=0.0001$), ויותר דזמין באזור האוטם ($p=0.0002$) לעומת חיות הביקורת. בחיות שקיבלו IGF נמצאה כי DNA laddering מופחת באזור האוטם בהשוואה לחיות הביקורת ($p<0.05$).

מסקנות. שני הפקטורים IGF-I ו- II שיפרו את תפקוד שריר הלב במנוחה. בתנאי עקה פרמקולוגית נראה כי הרזרבה התפקודית של החיות שטופלו ב-IGF-I הייתה טובה יותר מאלה המטופלות ב-IGF-II ולשתי הקבוצות טובה מקבוצת, הביקורת. שיפור בתפקוד שריר הלב היה מלווה בשימור המבנה של תאי שריר הלב וצפיפות כלי דם באזור האוטם ובאזור גבול האוטם.

תוכן עניינים