COMPARISON OF TWO WAYS OF ADMINISTRATION OF GROWTH FACTORS (bFGF AND RGTA) TO IMPROVE LATISSIMUS DORSI MUSCLE FLAP VASCULARIZATION AND TROPHICITY

Gilbert Zakine[1,2], MD, PhD, Christiane Ermisch[3], MD, Bertrand Loaille[4], MD, Paul Fornes[2], MD, Marc Sapoval[4], MD, PhD, José Courty[5], PhD, Jean Pierre Caruelle[5], PhD, Denis Barritault[5], PhD, Alain Carpentier[2], MD, PhD, Juan Carlos Chachques[2], MD, PhD

1/ Department of Plastic Reconstructive and Aesthetic Surgery, Burn Center, Rothschild Hospital, Paris, France
2/ Department of Cardiovascular Surgery, Broussais and Pompidou Hospitals, Paris, France
3/ Beethoven-Klinik, Cologne, Germany
4/ Department of Radiology, Pompidou Hospital, Paris, France
5/ Laboratory CRRET, UPRESA CNRS 753, University of Paris 12, France

Abstract

Background: In recent clinical studies, growth factors have been used in both cardiovascular and plastic surgery. We showed in a previous study that local administration of bFGF (basic Fibroblast Growth Factor), VEGF (vascular endothelial growth factor) and RGTA (regenerating agent), at the Latissimus Dorsi Muscle (LDM)-epicardial interface in a model of dynamic cardiomyoplasty, improved the vascularization and the trophicity of the LDM. In this study we compared two different methods of growth factor administration.

Methods: Right and left LDM flaps were performed in 24 sheep and left "in situ" in the thoracic wall. To create an ischemic environment, the serratus major was removed and the fascia was burned by electrocoagulation. Two different methods of growth factors administration were used: topically on the LDM surface through a multiperforated catheter for weekly administration over a 1-month period, or intramuscularly (IM) during the procedure. Basic fibroblast growth factor (n=12) and regenerating agent (n=12) a synthetic molecule which potentiates the effect of heparin binding growth factors, were administered. Each animal has been operated on both right and left sides: one side treated by one of the two factors, and the other as the control group. At three months, angiographic, histologic and histomorphometric studies were performed.

Results: Hypervascularization due to the development of new vessels has been demonstrated by angiographic studies of the animals treated with growth factors. Histomorphometric and histologic studies showed a significant increase in the number of capillaries and arterioles (100 fields/muscle) in the groups treated with bFGF (IM: 661.4 ± 43.2, catheter: 631.6 ± 72.1) and RGTA (IM: 563.1 ± 80.6, catheter: 574.8 ± 44.3), in comparison with the control group: 388.5 ± 74.3. The atrophy score was lower in the group treated with bFGF (IM: 0.91 ± 0.14, catheter: 0.87 ± 0.23) and RGTA (IM: 0.85 ± 0.33, catheter: 1.13 ± 0.38) than in the control group (1.65 ± 0.37).

Conclusions: RGTA and bFGF increase muscle vascularization and avoid muscle atrophy in an experimental model of LDM flap. A single intramuscular injection during surgery which is as efficient as weekly administration through a catheter, is the simpler and the better method for growth factor administration.

Key words: Latissimus dorsi muscle flap – cardiomyoplasty – growth factors – heart failure.

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Introduction

The aim of this study was to evaluate the effects of local administration of growth factors in a LDM flap experimental model by two different methods: topically at the muscle surface through a catheter, or intramuscularly.

In a preliminary study [30] we have evaluated the effects of three different factors: bFGF, VEGF and RGTA, in an experimental model of cardiomyoplasty. This surgical procedure in which the electrostimulated LDM is wrapped around the heart, is indicated in severe chronic heart failure refractory to pharmacological therapy, and has the advantage of not requiring a donor heart and of low financial costs. Several reports on cardiomyoplasty demonstrated good long-term results in approximately 70% of cases [2,4,8,10,15,17]. In the remaining 30%, it appears that the efficacy of the procedure is reduced, due to several causes; the most important is partial ischemia of the distal part of the LDM resulting in partial atrophy.

In the present study we evaluated the effect of the administration of growth factors (bFGF and RGTA) by two different methods: 1) administration through a catheter at muscle deep surface, and, 2) injection into the LDM. Both latissimus dorsi muscles of each animal were dissected, afterwards the effects of the two different methods of administration were compared.

Method

Twenty-four sheep (mean weight of 35.5 ± 6 kg) underwent dissection of the left and right LDM flap. All procedures were performed under general anesthesia. Animals were premedicated with 5 mg/kg IM acepromazine, and anesthesia was induced with 8 mg/kg IV propofol, and maintained with isoflurane 1-2% followed endotracheal intubation.

The LDM flap was completely dissected and collateral vessels were electrocoagulated or ligated, thus the only vascular supply arises from the thoraco-dorsal pedicle. To create an ischemic environment, the serratus major was removed and the fascia was electrocoagulated. In group 1, a catheter was positioned on the deep surface of the LDM and connected to a subcutaneous port chamber. On the left LDM we administered one of the two factors and in the right LDM we administered the control solution, so that each sheep became its own control. Injections through the port chamber were performed on the operative day and weekly for one month. In group 2, a single intramuscular injection was performed after flap dissection.

Growth factor administration was performed using a subcutaneous chamber connected to a multiperforated catheter with 5 injection holes (Poly- sites 1007 ISP, LPI, France), positioned during surgery under the LDM (Fig.1). Factors were injected into the port chambers on postoperative day 1, 7, 14 and 21.

FIGURE 1. The growth factors delivery catheter was placed beneath the LDM.

The quantity of each factor administered was calculated for optimal efficacy according to the mean weight of the sheep LDM (220+/-26 g). The volume of injection was 5 ml for each sheep and the dose administered was 80 µg of bFGF, and 480 µg of Regenerating Agent. In the control group, PBS (Phosphate Buffered Saline) used for dilution of the growth factors, was administered in an identical manner as described for growth factor administration.

In groups in which the factors were administered intramuscularly, one injection was performed after the LDM flap dissection in the middle and distal part of the muscle.

Evaluation

Muscle trophicity and vascularization were evaluated by histological, histomorphometric and angiographic studies.

Angiographic Studies

At the end of the third post-operative month, angiographic studies were performed under general anesthesia in order to evaluate LDM vascularization. Opacification of the thoracodorsal artery was performed via catheterization of both axillary arteries. All angiograms were performed using the same contrast media, injected at the same rate using the same catheter, after selective catheterization of the thoracodorsal artery. All images were realized in the same angiographic device (Siemens Angiostar) using a 1024 x 1024 matrix in a 16 cm field of view. Images were printed on 12 x 12 cm hard copies, including at least one image from the early phase (good opacification of the major vessels), middle phase (good visualization of major and smaller vessels), and late phase (best opacification of smallest vessels).

Two independent observers, blinded to the treatment administered conducted image assessment. Helical and tortuous vessels with little or no bifurcations were defined as neovessels. A semi-quantitative scale was used

to create the following score: grade 0: no neovessels; grade 1: some neovessels; grade 2: numerous neovessels. The visualization of a tissue blush on the late phase was evaluated using the following tissue perfusion score: grade 0: no blush, grade 1: mild blush; grade 2: major blush. Blush refers to capillaries that are below the spatial resolution of the system and cannot be seen as individual vessels but reflect increased tissue perfusion. For statistical analysis, the mean between the two observers was considered as the final value and used for comparisons between the groups.

Histopathological Studies

Following angiographic studies, all animals were euthanized after injecting heparin, papaverine, and formaldehyde (into the left axillary artery). In addition, a 4% formaldehyde solution was perfused into the LDM mass through the thoracodorsal artery, at the animal's in vivo calculated main blood pressure. Papaverine was used to obtain a physiologic vasodilatation, in order to better quantify vascularization and more objectively compare the animals.

Following in situ formaldehyde perfusion, the LDM was excised and put in a 10% formaldehyde solution for further fixation. After fixation 7 equally distant slides were obtained from the mid to the distal portion of the LDM, so that the same portion of the muscle was examined in all animals. The 7 slides were embedded in paraffin. Each paraffin bloc was cut into 3, 3 µm thick sections and stained with H E S (hematoxylin-eosin-saffron), Gordon Sweet and orcein. HES is a stain routinely used to visualise cells and collagen network, Gordon Swett is used for reticulin fibers surrounding cells and vessels, and orcein is used to detect elastin fibers present in vessels.

Histomorphometry was used to count the numbers of vessels and to quantify the area occupied by muscle cells in each histological slide.

Histological slides were examined under blind conditions by a cardiovascular pathologist (PF). We analyzed 14 or 15 contiguous fields per histological slide at a X 250 magnification (i.e. a total of 100 field for the 7 slides per sheep). The number of fields was chosen to obtain a thorough examination of the muscle, from its mid to distal part.

In pathology, muscular atrophy is defined by a decrease in size of muscle cells (myocytes) over time resulting in their loss. These lost cells are progressively replaced by fibrosis and / or adipocytes. Grossly, the process results in a decrease in muscle thickness; physiologically, it results in a decrease in performance. In order to quantify the atrophic process, we evaluated the relative area occupied by myocytes in each slide (%). A score of 0 to 3 was attributed according to the degree of atrophy. As we done for the vessel counts, the 7 slides were analyzed and the mean of the degree of atrophy was calculated. The following muscle atrophy score was created: 0: no atrophy; 1: when the relative area occupied by myocytes per slide was more than 2/3; 2: between 2/3 and one third; and 3: when myocytes occupied less than one third of the total slide area.

Statistics

An analysis of variances (ANOVA) parametric test, followed by the Newman-Keuls multiple comparison test was used for comparison of the mean number of capillaries and arterioles per group. For the atrophy score and the angiographic grading, comparisons were made by the Kruskal-Wallis non parametric test followed by Dunnís multiple comparison test. Results are expressed as mean values (s.e.m: standard error of the mean).

Results

Angiographic studies

A score of neovessels and a score of blush tissue were used to evaluate neovascularization.

The mean neovessels scores were; for the bFGF group: 1.83 ± 0.27 ($p < 0.05$), for the RGTA group: 1.66 ± 0.44 ($p < 0.01$), and for the control group: 0.33 ± 0.44.

The mean tissue blush scores were; for the bFGF group: 1.66 ± 0.44 ($p < 0.05$), for the RGTA group: 1.66 ± 0.44 ($p < 0.05$), and for the control group: 0.16 ± 0.12.

All animals treated with bFGF or RGTA demonstrated hypervascularisation due to neoangiogenesis. Helical arteries, tissue blush and neoanastomoses, which are neovascular structures, were observed.

Histologic studies

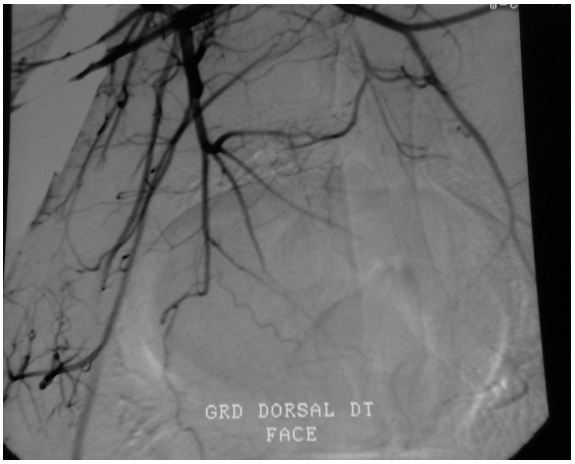
The mean atrophy score of the 7 sections was used for quantification of muscle atrophy for each sheep. Results were; for the bFGF-IM group: 0.91 ± 0.14 ($p<0.05$), for the bFGF-catheter group: 0.87 ± 0.23 ($p<0.05$), for the RGTA-IM group: 0.85 ± 0.33 ($p<0.05$), for the RGTA-catheter group: 1.13 ± 0.38, and for the control group: 1.65 ± 0.37. Scores were defined as 1 (absence of atrophy) to 3 (maximum atrophy). A lower atrophy score was observed in all groups treated with growth factors, however results were not statistically significant between the different treated groups. (Table 1 and Fig. 2)

TABLE1: Results of histomorphometric and histological studies.

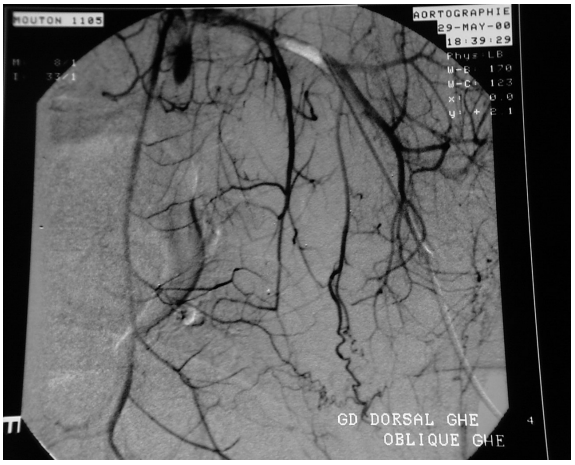
Groups	Number of vessels/100 Fields	Atrophy score
Control	388.5 +/- 74.3	1.65 ± 0.37
bFGF-IM group	661.4 ± 43.2 ($p<0.05$)	0.91 ± 0.14 ($p<0.05$)
bFGF-Catheter group	631.6 ± 72.1 ($p<0.05$)	0.87 ± 0.23 ($p<0.05$)

RGTA -IM	563.1 ± 80.6	0.85 ± 0.33
	(p<0.05)	(p<0.05)
RGTA-catheter	574.8 ± 44.3	1.13 ± 0.38
	(p<0.05)	(p<0.05)

FIGURE 2: Angiographic studies
A: Control group.



B: RGTA group showing hypervascularization of thoracodorsal artery branches and helical arteries (neoangiogenesis).

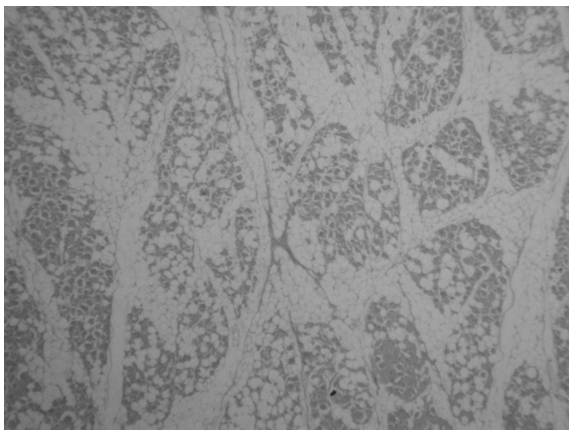


Histomorphometric studies

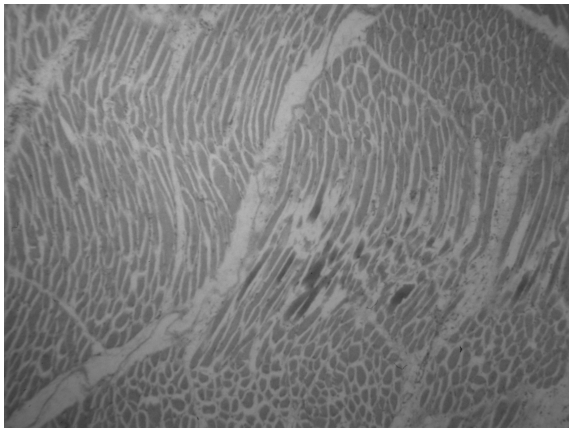
An increase in the number of capillaries and arterioles, calculated for a defined area (100 fields / muscle) was observed in the groups treated with growth factors. The neovessels score was higher in bFGF group treated by intramuscular injection: 661.4 ± 43.2 or through catheter: 631.6 ± 72.1 and RGTA group by intramuscular injection: 563.1 ± 80.6 and through catheter: 574.8 ± 44.3, in comparation with the control group: 388.5 ± 74.3. The atrophy score was lower in the group treated with bFGF (IM: 0.91 ± 0.14, catheter: 0.87 ± 0.23) and RGTA (IM: 0.85 ± 0.33, catheter: 1.13 ± 0.38) than in the control group (1.65 ± 0.37).

Results were statistically significant for all the treated groups (Table 1 and Fig. 3).

FIGURE 3: Histological studies
A: Control group showing atrophy of the LDM (hematoxilin-eosin-saffron, magnification x40).



B: RGTA group showing a preserved LDM trophicity (hematoxilin-eosin-saffron, magnification x40).



Discussion

The purpose of this study was to evaluate the method of growth factor administration which best preserve trophicity and improves vascularization of the LDM flap.

Growth factors are polypeptides, which stimulate the proliferation, the migration and the differentiation of cells.

BasicFGF, present in the majority of tissues is essential for the proliferation and differentiation of meso and ectodermic cells and plays an important role in the regulation of vascularization.

RGTA, which is not an endogenous growth factor but an original product, synthetized by successive substitution of a dextran molecule, possesses heparin or heparan sulfate properties (no anticoagulation effect) by protecting growth factors from pH, thermal or proteolytic denaturation [27]. Consequently, RGTA increases the activity of "Heparin-Heparan binding" growth factors. RGTA may improve the action of FGF, VEGF, TGF (Transforming Growth Factor), PDGF (Platelet Derived Growth Factor), or BMP (Bone Morphogene Protein). RGTA has demonstrated an ability to accelerate and optimize healing in different experimental models [3,11,19].

Growth factors have been applied in both cardiovascular, and plastic surgery. In ischemic cardiomyopathies, bFGF injected around the distal anastomosis of coronary artery bypass grafts increased neoangiogenesis in the patient treated group [25]. In another clinical study, bFGF was administered using a microcapsule, implanted in proximity of ischemic myocardial areas [26]. In an experimental porcine model of chronic myocardial ischemia, bFGF was injected into the pericardial sac. The results of this study demonstrated an improvement in myocardial perfusion and function in the ischemic territory and increased myocardial vascularity [16]. In a recent experimental study, biodegradable hydrogel microspheres with bFGF improved left ventricular function in pig with chronic myocardial infarction [24]. In chronic limb ischemia, a gene coding for the Vascular Endothelial Growth Factor (VEGF) has been injected intra-arterially increasing distal revascularization in a group of patients presenting arteriosclerotic disease [14]. In burn patients, local (topical) application of bFGF accelerated re-epidermization [12] and in chronic wounds, several growth factors have been used with success [23].

In a previous study on experimental cardiomyoplasty, Mannion et al [18] showed that the administration of bFGF into the left subclavian artery preserves vascularization and trophicity of the LDM. We believe that intra-arterial administration of growth factors, by increasing proliferation and migration of cells, may present risks, with consequent systemic effects. Furthermore, successive intravascular administration is technically difficult and costly. Isner et al [14] administered VEGF via the femoral arteries in order to stimulate collateral circulation in patients presenting an obliterative arteriopathy of the lower limbs. Some patients developed painful and hemorrhagic angiomas and for this reason, in the majority of recent clinical studies, growth factors are administered locally. Schumacher et al [25] injected bFGF directly into the myocardium in order to revascularize ischemic areas around coronary anastomoses. Vale et al [28] performed therapeutic angiogenesis using catheter-based myocardial gene transfer (encoding VEGF-2) guided by electromechanical mapping.

In an experimental model, gene transfection for human hepatocyte growth factor (hHGF) combined with cellular cardiomyoplasty was used to regenerate the impaired myocardium. The liposome-plasmid complex (including 15 μg of the human HGF cDNA) was injected directly into the infarcted area [20]. More recently recombinant human vascular endothelial growth factor, which was injected in intracoronary and intravenously in patient with stable angina, was well tolerated and resulted by day 120 in significant improvement in angina and quality of life [13].

Concerning the dynamic cardiomyoplasty surgical technique, another interesting approach to improve LDM irrigation before CMP is to use a vascular delay procedure or electrostimulation before harvesting the muscular flap, thus improving blood flow in the distal portion of the LDM [1,5,9,29]. The vascular delay procedure consists of ligation of the intercostal perforating vessels between the LDM and the chest wall, one month before complete elevation of the LDM. This experimental approach was also associated with intraarterial administration of growth factors [6].

In order to propose growth factor therapy for clinical CMP and chose the best *methods for administration*, we have compared two different modes of growth factor administration, using a multiperforated catheter positioned at the LDM deep face and connected to a subcutaneous port chamber, or directly injected in the LDM. Histomorphometric studies permitted precise evaluation of the neoangiogenesis. An increased number of capillaries and arterioles was observed in all the groups treated with growth factors.

The LDM muscle flap has one of the best vascular supplies. In effect, the thoraco-dorsal artery has a large diameter and the ischemic complications in reconstructive surgery are uncommon. This flap has been chosen in our study because it is used in cardiomyoplasty, in which the efficacity of the procedure can be reduced due to several causes: chronic electrostimulation or cardiac insufficiency, leading to low perfusion of tissues and organs [7,21,22].

In summary, this study demonstrates that in an experimental LDM flap model, local administration of growth factors appears efficient. A statistically significant correlation between the number of capillaries and arterioles and the amount of muscular atrophy was observed. A single intramuscular injection seems to be a simple and safe way of administration.

Conclusion

Preservation of LDM trophicity was due to the action of growth factors on neovascularization and myogenesis. The results presented above are promising for both muscle and myocutaneous flaps used in the clinical setting of cardiomyoplasty and plastic and reconstructive surgery. The two local growth factor delivery approaches used in our study appear efficient, but a single injection in the muscle seems to simplify the procedure.

We believe that this approach may improve muscle morphology and function, optimizing functional results following cardiomyoplasty procedures. Moreover, this approach should avoid muscle flap ischemic complications in plastic and reconstructive procedures.

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Address correspndence to:

Dr Zakine, Department of Plastic Reconstructive and Aesthetic Surgery, Burn Center, Rothschild Hospital, Paris, France; e-mail: zakinegilbert@yahoo.fr.

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