Angiogenic effect of intramuscular administration of basic and acidic fibroblast growth factor on skeletal muscles and influence of exercise on muscle angiogenesis

A Efthimiadou, B Asimakopoulos, N Nikolettos, A Giatromanolaki, E Sivridis, D N Papachristou, E Kontoleon

Background: Angiogenic factors which control the angiogenic process represent a promising strategy for restoration of blood flow, but require further evaluation before clinical use. Exercise has also been reported to induce neoangiogenesis in muscles.

Objectives: To evaluate the angiogenic effects of basic fibroblast growth factor (b-FGF) and acidic fibroblast growth factor (a-FGF) on rat gastrocnemius muscle, when administered intramuscularly, and to compare them with those obtained by daily exercise.

Methods: Forty-nine rats were allotted to the following groups: A, controls; B, exercise by swimming; C1 and C2, intramuscular injection of b-FGF and a-FGF respectively; D1 and D2, b-FGF and a-FGF injection in combination with exercise. The antibody mouse anti-rat CD31 was used to evaluate the numbers of blood vessels present in histological preparations of gastrocnemius muscle.

Results: Significant increases in the numbers of blood vessels of the right gastrocnemius muscles in groups C1 and D1 were observed compared with controls (p<0.05). There was only a slight increase in angiogenesis in the left gastrocnemius muscle of groups C1 and D1 compared with controls (p>0.05), and there was a decrease in angiogenesis in the gastrocnemius muscle of the swimming group compared with controls.

Conclusion: The intramuscular administration of b-FGF, but not a-FGF, induced significant local angiogenesis in gastrocnemius muscle at the site of injection.

Materials and Methods

Design

Forty-nine male Wistar rats (330–400 g body weight; age 4 months) were used for this study. During the experimental period, the animals lived under stable conditions of temperature and a reverse light cycle programme, and were allowed to eat ad libitum. They were divided into six groups (table 1).

Group A, consisting of 14 rats, were controls. This group was subdivided into: A1, seven rats to be compared with the swimming group; A2, seven rats in which 0.1 ml saline was injected via a syringe of insulin under ether anaesthesia into the neck and allowed to eat ad libitum. They were divided into six groups (table 1).

Abbreviations: α-FGF, acidic fibroblast growth factor; β-FGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor
the middle of the right gastrocnemius muscle every three days for 15 days.

Group B consisted of seven rats that swim in water of constant temperature (36°C) every day for 15 days. The duration of the swim was five minutes on the first day and this increased progressively up to 60 minutes on day 10, after which it remained constant until the end of the experiment.6

Group C1 consisted of seven rats that received 1 µg b-FGF injected via a syringe of insulin into the middle of the right gastrocnemius muscle under ether anaesthesia every three days, for 15 days. Group C2 consisted of seven rats that received an equimolar dose of a-FGF (0.88 µg) instead of b-FGF according to the same protocol.

Group D1 consisted of seven rats that received b-FGF as described for group C1 but in combination with swimming as described for group B. Group D2 consisted of seven rats that received a-FGF as in group C2 but in combination with swimming as in group B.

The rats were killed on the 18th day, and the gastrocnemius muscles from both legs were removed, weighed, and sent for histological examination.

**Histological examination**

The tissues were fixed in 10% formalin, embedded in paraffin, cut transversely into 3 µm thick sections in the middle of the specimen, and studied by immunohistochemical techniques.4 To evaluate angiogenesis, blood vessels were detected by the use of a monoclonal mouse anti-rat antibody (CD31, clone: PECAM-1; Dako, Glostrup, Denmark), which recognises the surface antigen CD31 of endothelial cells of mice. The immunohistochemical method of alkaline anti-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Experimental groups of rats and their treatments</th>
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<tbody>
<tr>
<td>Group</td>
<td>Treatment</td>
</tr>
<tr>
<td>A1</td>
<td>No treatment (controls)</td>
</tr>
<tr>
<td>A2</td>
<td>Intramuscular saline (controls)</td>
</tr>
<tr>
<td>B</td>
<td>Swimming</td>
</tr>
<tr>
<td>C1</td>
<td>Intramuscular b-FGF</td>
</tr>
<tr>
<td>C2</td>
<td>Intramuscular a-FGF</td>
</tr>
<tr>
<td>D1</td>
<td>Intramuscular b-FGF + swimming</td>
</tr>
<tr>
<td>D2</td>
<td>Intramuscular a-FGF + swimming</td>
</tr>
</tbody>
</table>

α-FGF, acidic fibroblast growth factor; b-FGF, basic fibroblast growth factor.

Statistical analysis

The following variables were analysed: body weight at the beginning and end of the experiment; weight of each gastrocnemius muscle (left and right leg); number of blood vessels per optical field in each gastrocnemius muscle. Descriptive statistics were calculated for all groups. Comparisons among groups were performed with the Mann-Whitney U test and Kruskal-Wallis test using the Statistica 4.5 statistical package for Windows (StatSoft Inc, Tulsa, Oklahoma, USA). p < 0.05 was considered significant.

**RESULTS**

**Effects of exercise, b-FGF, and a-FGF on angiogenesis in gastrocnemius muscles**

There were no deaths from the repeated anaesthesia. Also no difference in angiogenesis was observed in the gastrocnemius muscles between the control groups A1 and A2 (table 2, fig 1A).

Comparison between groups A1 and B revealed a significant reduction in the number of vessels in both right (p < 0.05) and left (p < 0.05) gastrocnemius muscles triggered by exercise (table 2, fig 1B). On the other hand, comparing the muscle weights between group A1 and group B (table 3), a significant increase in the weight of both the left (p < 0.01) and right (p < 0.01) muscle was triggered by exercise.

In group C1, the mean number of blood vessels was different in the right gastrocnemius muscle, where b-FGF was injected intramuscularly, from the left gastrocnemius muscle (table 2). Comparison of group C1 with controls (group A2) with regard to the vessels in the right and left gastrocnemius muscle reveals that b-FGF increased vascularisation significantly (p < 0.05) and selectively only in the right muscle (table 2, fig 1C). Furthermore, in group C1, the number of vessels in the right and left muscles was significantly higher than in the muscles of group B (p < 0.005 and p < 0.05 respectively).

It was also observed that the number of vessels in the right muscle in group C2 was significantly higher than in group B (p < 0.05) but not higher than in group A2 (table 2, fig 1D). However, groups C2 and B did not show any differences in angiogenesis in the left gastrocnemius muscle.

The combined administration of b-FGF and swimming (group D1) resulted in different effects on the right and left gastrocnemius muscles (table 2). Compared with group A2, in group D1 vascularisation was significantly increased in the right muscle (p < 0.05) but not in the left one (fig 1E). Similarly, the vessels in the right and left muscles of group D1 were significantly increased compared with those of group B (p < 0.005 and p < 0.05 respectively).

In group D2, it was also observed that the number of vessels in only the right muscle, and not the left one, was significantly higher than in group B (p < 0.05) (table 2, fig 1F).

Swimming appears to significantly decrease the vascularisation of both right and left gastrocnemius muscles compared with controls, probably as a result of changes in muscle mass. The intramuscular injection of b-FGF significantly increased vascularisation locally in the right muscle into which it was injected, whereas the intramuscular injection of a-FGF did not result in vascularisation of the injected muscle. When there was simultaneous administration of angiogenic factors and swimming, only b-FGF caused a major local increase in vessels in the muscle injected. No angiogenic action of either b-FGF or a-FGF was observed on the left side perhaps because of the predominance of the effects of exercise.
Effects of exercise, b-FGF, and a-FGF on the weight of rat gastrocnemius muscles

The weights of the right and left gastrocnemius muscles were also determined at the end of the experiment as an index of muscle mass (table 3). Exercise (group B) significantly increased the weight of the right (p<0.01) and left (p<0.01) muscles compared with controls. The same significant increase in the weight of both the right (p<0.01) and left (p<0.01) muscles was observed in group C1 (intramuscular b-FGF) and group D1 (intramuscular b-FGF plus swimming) compared with controls.

DISCUSSION

Angiogenesis, the creation of new vessels from pre-existing ones, is a complex process initiated under various physiological (embryonic growth, exercising muscles, menstrual cycle) and pathological (tumours, ischaemia, etc) conditions. Although the exact mechanisms underlying angiogenesis are

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of vessels per optical field</th>
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<tbody>
<tr>
<td></td>
<td>Left muscle</td>
</tr>
<tr>
<td>A1</td>
<td>16.94 (2.80)</td>
</tr>
<tr>
<td>A2</td>
<td>17.00 (3.80)</td>
</tr>
<tr>
<td>B</td>
<td>12.70 (3.00)*</td>
</tr>
<tr>
<td>C1</td>
<td>18.40 (3.30)*</td>
</tr>
<tr>
<td>C2</td>
<td>15.70 (2.98)</td>
</tr>
<tr>
<td>D1</td>
<td>17.00 (1.10)*</td>
</tr>
<tr>
<td>D2</td>
<td>16.20 (1.87)</td>
</tr>
</tbody>
</table>

Values are mean (SD).
*p<0.05 compared with controls.
<table>
<thead>
<tr>
<th>Group</th>
<th>Number of vessels per optical field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left muscle</td>
</tr>
<tr>
<td>A1</td>
<td>2.13 (0.15)</td>
</tr>
<tr>
<td>A2</td>
<td>2.18 (0.14)</td>
</tr>
<tr>
<td>B</td>
<td>2.67 (0.23)*</td>
</tr>
<tr>
<td>C1</td>
<td>2.92 (0.24)*</td>
</tr>
<tr>
<td>C2</td>
<td>2.50 (0.16)</td>
</tr>
<tr>
<td>D1</td>
<td>2.56 (0.12)**</td>
</tr>
<tr>
<td>D2</td>
<td>2.49 (0.18)</td>
</tr>
</tbody>
</table>

Values are mean (SD).
*p<0.01, **p<0.05 compared with controls.
A1, No treatment; A2, 0.1 ml saline injected into the right gastrocnemius muscle every three days for 15 days; B, made to swim every day for 15 days; C1, 1 µg basic fibroblast growth factor (b-FGF) injected into the right gastrocnemius muscle every three days, for 15 days; C2, an equimolar dose of acidic fibroblast growth factor (a-FGF; 0.88 µg) injected into the right gastrocnemius muscle every three days, for 15 days; D1, injected with b-FGF in combination with swimming; D2, injected with a-FGF in combination with swimming.
It is known that receptors for fibroblast growth factors (FGFs) exist in organs such as brain, heart, and muscle. Also, several studies have shown that FGFs have an angiogenic effect in pathological conditions, such as cancer but also under ischaemic conditions—for example, in the cardiac muscle.

In this study, we injected b-FGF and a-FGF intramuscularly into the right gastrocnemius muscle. We observed a local angiogenic effect with b-FGF but the effect of a-FGF was negligible. Also exercise reduced the number of vessels per optical field in the skeletal muscles. As our method detects vessels per optical field, the only explanation of the above results is that increased muscle mass led to the artificial result of a reduction in angiogenesis. From the results of angiogenesis when FGF administration was combined with exercise, we can conclude that only b-FGF led to a local increase in angiogenesis in the injected muscle, with a-FGF only having slight local effect.

In conclusion, this study shows that intramuscular injection of b-FGF but not of a-FGF is effective in skeletal muscle only locally and at high concentrations. These observations are interesting with regard to the potential administration of b-FGF to alleviate conditions characterised by insufficient blood supply, such as limb ischaemia. Also, the study provides a basis for further application of b-FGF in humans for the treatment of ischaemic vascular lesions.

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Competing interests: none declared

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COMMENTARY

This is a basically sound paper that concerns the investigation of whether intramuscular administration of b-FGF and a-FGF can result in muscle angiogenesis, and whether any increase in angiogenesis caused by FGF administration is similar to the angiogenesis induced by exercise training. A statistically significant increase in angiogenesis resulted from intramuscular injection of b-FGF, but not from a-FGF injection or exercise alone. These observations may be of great importance in humans for the clinical treatment of ischaemic vascular lesions and for conditions characterised by insufficient blood supply.

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