Glycosaminoglycan mimetics (RGTA) modulate adult skeletal muscle satellite cell proliferation in vitro

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Abstract: Muscle regeneration occurs through the activation of satellite cells, which are stimulated to proliferate and to fuse into myofibers that will reconstitute the damaged muscle. We have previously reported that a family of new compounds called “regenerating agents” (RGTAs), which are polymers engineered to mimic heparan sulfates, stimulate in vivo tissue repair. One of these agents, RG1192, a dextran derivative substituted by CarboxyMethyl, Benzylamide, and Sulfate (noted CMBS, RGTA type), was shown to improve greatly the regeneration of rat skeletal muscle after severe crushing, denervation, and acute ischemia. In vitro, these compounds mimic the protecting and stabilizing properties of heparin or heparan sulfates toward heparin-binding growth factors (HBGFs). We hypothesized that RGTA could act by increasing the bioavailability of some HBGF involved in myoblast growth and thus asked whether RGTA would alter the ability of satellite cells to proliferate. Its effect was tested on primary cultures of rat satellite cells. The RG1192 stimulated the proliferation of satellite cells in vitro in a dose-dependent manner. It appeared to be as efficient as natural glycosaminoglycans (GAGs; heparan sulfate, dermatan sulfate, or keratan sulfate) in stimulating satellite cell proliferation but was about 100 times more efficient than heparin. RG1192 stimulated satellite cell proliferation by increasing the potency of fibroblast growth factor 2 and scatter factor–hepatocyte growth factor. It also partially restored myoblast proliferation of satellite cells with chlorate-induced hyposulfation. Taken together, our results explain to some extent the improving effect of RGTA with a CMBS structure, such as the RG1192, on muscle regeneration in vivo by providing support for the hypothesis that RGTA may act by increasing the potency of some HBGFs during the proliferation phase of the regenerating muscle. © 2002 Wiley Periodicals, Inc. J Biomed Mater Res 62: 46–55, 2002

Key words: satellite cells; regenerating agents; heparan sulfate mimetics; growth factor; fibroblast growth factor; scatter factor–hepatocyte growth factor; glycosaminoglycan

INTRODUCTION

Skeletal muscle cell differentiation is a process whereby mesoderm stem cell-derived myoblasts exit from cell cycling and acquire the biologic and morphologic phenotype of myoblasts and fused myotubes. The final differentiation phenotype is associated with innervation of the myofibers. The capacity of myoblasts to proliferate or differentiate is determined by cell–cell contact, cell–extracellular matrix (ECM) interaction, and growth factors. Growth factor effects are mediated through their binding both to high- and to low-affinity receptors, which are mainly heparan sulfate proteoglycans (HSPGs). It is becoming increasingly clear that growth factor activities can be controlled by their association with ECM, and that cells can interpret the growth factor signal in the context of the surrounding matrix.

A number of growth factors found in skeletal muscle are believed to control various aspects of muscle organogenesis, growth, and regeneration after damage. The list of growth factors known to regulate myoblast proliferation and differentiation includes the heparin-binding growth factors (HBGFs), such as fibroblast growth factors (FGFs), the scatter factor–hepatocyte growth factor (SF-HGF), and the transforming growth factor-β (TGF-β) families (see review2–4), whereas others such as IGF-I and -II (insulin-
like growth factors) are regulated by their binding proteins, which also bind heparin.5–9 Among the growth factors known to control myoblast proliferation, FGF2 is well known to play a pivotal role. Studies concerning this growth factor’s effect on myoblasts are the most documented.10–18 The SF-HGF, which plays a crucial role in muscle development, has also been shown to be produced by fibers in wounded muscle and to control satellite cell proliferation.17,19–23

We synthesized several chemically modified dextrans obtained by successive substitution of carboxymethyl (CM) groups, followed by benzyl amidation (B), and finally O-sulfonation (S), noted as CMBS. Molecules with a CMBS structure have been selected for their capacity to interact with and protect several HBGFs such as FGF1 and 2 or TGF-β against proteolysis.24,25 Thus, these molecules mimic some of the protecting and potentiating properties of heparin or heparan sulfate with regard to growth factors. In addition, these dextran derivatives have been shown to inhibit proteolytic activity of several proteases, such as plasmin,26 leukocyte elastase,25 or calpain (Ledoux, 2001, personal communication). In vivo, these molecules are potent activators for repair of different tissues, such as skin,27 bone,28,29 colonic30,31 and corneal32 defects, or heart stroke.33 They also highly improve and stimulate skeletal muscle regeneration after crushing,34–37 or muscle ischemia and denervation.38

Muscle regeneration occurs through the activation of undifferentiated myoblasts called satellite cells, which are located between the basal lamina and the plasmalemma of skeletal muscle fibers. These cells, first described in amphibians,39 are responsible for muscle fiber growth during development.40,41 They also allow muscle fiber repair after myolysis caused accidentally or by genetic defects such as muscular dystrophy.41 Satellite cells play a pivotal role in muscle regeneration, and considering the in vivo improvement of this process by a glycosaminoglycan (GAG) mimetic, RG1192,34–37 we investigated whether satellite cells might be a target for this drug. We therefore compared the effects of RG1192 to those obtained with the natural GAGs, as well as several compounds that are intermediate steps of the RG1192 synthesis. Our major aim was to find a rationale to explain both the in vivo effects and the relationship between RGTA structure and functions.

MATERIALS AND METHODS

Materials

Experimental animals were 2-month-old, male Wistar rats provided by Ifa Credo. Dulbecco’s Modified Eagle’s Medium (DMEM) and fetal bovine serum were from Gibco BRL, and horse serum from Boeringher. Plates were from Nunc. Tritium-labeled thymidine was from ICN. Heparin was a kind gift from M. Petitou from Sanofi (France); its molecular weight was estimated at 16 kDa. Other products, such as dextran sulfate, gelatin, heparan sulfate (HS), dermatan sulfate (DS), keratan sulfate (KS), pronase, and chlorate, were from Sigma-Aldrich (France).

Recombinant FGF2 was prepared in our laboratory. SF-HGF was from conditioned medium of carcinoma bladder cells (NBTII) provided by Dr. J. Jouanneau (URA1337 CNRS, Paris, France). The presence of SF-HGF in conditioned medium was checked using the scattering effect test on Madin–Darby Canine Kidney (MDCK) cells, as described by Stoker and Perrymen.42 The preparation produced a scattering effect up to a dilution 1/80. Antibodies raised against FGF2 and SF-HGF were from R&D.

Dextran derivatives

The different molecules of dextran derivatives used and their characteristics are shown in Table I. The different steps of synthesis and structural analysis were recently published by Ledoux et al.26 In brief, T40 dextran was substituted by carboxymethylated (RG1100), or by carboxymethylation followed by amidation with benzylamine (RG1106), followed by O-sulfonation (RG1192). The structures of the molecules are shown schematically in Figure 1, and their composition and molecular weights are listed in Table I.

Methods

Satellite cell primary cultures

Myogenic satellite cells were dissociated from fibers of limb muscles with pronase, as previously described.11,43,44 Cells were seeded (2000 cells/cm²) on dishes coated with 0.1% gelatin and grown continuously in DMEM containing 1 g/L glucose, 10% fetal bovine serum, and 10% horse serum. Cultures were grown in 5% CO₂ at 37°C for up to 12 days. Culture medium was changed at day 3 unless otherwise specified.

Measurement of cellular proliferation

Cells seeded on 24-dish plates were washed twice with phosphate-buffered saline (PBS), dissociated by trypsin, and counted at the indicated times in a Coulter Counter® (Coultronics, France). Alternatively, cellular proliferation was measured by (³H)-thymidine incorporation into cells as described previously.43 In brief, cells at day 3 or 4 after seeding were starved for 24 h in the presence of 0.1% bovine serum albumin (BSA) or 0.25% fetal calf serum and 0.25% horse serum. The cultures were then treated with the 10% sera or RGTA and/or growth factor for a further 24 h.
Table I

Chemical Characteristics of Modified Dextrans (RGTA)*

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Type</th>
<th>CMb</th>
<th>Su</th>
<th>CMBn</th>
<th>H*</th>
<th>CM</th>
<th>Su</th>
<th>CMBn</th>
<th>Average M,</th>
</tr>
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<tbody>
<tr>
<td>RG192</td>
<td>CMBS</td>
<td>0.31</td>
<td>1.38</td>
<td>0.39</td>
<td>0.92</td>
<td>0.19</td>
<td>0.12</td>
<td>0.45</td>
<td>0.93</td>
</tr>
<tr>
<td>RG106</td>
<td>CMB</td>
<td>0.51</td>
<td>0.66</td>
<td>1.83</td>
<td>0.30</td>
<td>0.30</td>
<td>0.21</td>
<td>—</td>
<td>0.43</td>
</tr>
<tr>
<td>RG1100</td>
<td>CM</td>
<td>0.49</td>
<td>—</td>
<td>—</td>
<td>2.51</td>
<td>0.29</td>
<td>0.20</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*The chemical characterization of each polymer is represented by the degree of substitution (d.s.) of individual group per glucosidic unit. Each d.s. value is determined and confirmed by acidimetric titration, elementary analysis, and 1H NMR. A d.s. value of 3 represents the maximum substitution, because one glucosidic unit contains three reactive OH groups on C-2, C-3, and C-4 positions. The position of each group on the C-2 versus C-3 + C-4 positions was also determined by analyzing the anemic proton signal in 1H-NMR. In this representation, d.s. values of 1 and 2 represent the maximum substitution for C-2 and C-3 + C-4 positions, respectively. Standard deviations of d.s. values were less than 5% (n = 3).

thymidine was added for 6 h, 18 h after the medium change. Radioactivity was measured by liquid scintillation in cultures treated with trichloroacetic acid. Each experiment included nonstimulated cultures (0.1% BSA or 0.25% both fetal and horse sera) and cultures treated with both sera (10%). Maximum stimulation was the value of [3H]thymidine incorporation in the presence of both sera to which incorporation in the nonstimulated cultures was deducted. Results in treated cultures were sometimes expressed as percentages of the maximum of stimulation of [3H]thymidine incorporation.

In some experiments, cells were treated with conditioned medium of rat bladder tumor cell-like (NBTII) cultures diluted by half in DMEM. This conditioned medium contained SF-HGF, which produced a scattering effect up to a dilution of 1/64 in a biological test using MDCK cells.

Statistical analysis

To compare experimental values, we used the Student t test.

RESULTS

Effect of RGTA on satellite cell proliferation

Under our standard growth culture conditions, from day 3, the number of satellite cell per plate increased after seeding and began to fuse into multinucleated myotubes after day 7. Satellite cell cultures were treated just after seeding with RG1192 at the indicated concentrations (Fig. 2). The cell number scoring between days 4 and 6 after seeding showed that RG1192 modulated satellite cell proliferation in a dose-dependent manner. Between 0.1 and 10 μg/mL (i.e., between about 0.70 nM and 70 nM), a scored increase in proliferation reached its maximum in the presence of 1 μg/mL (7 nM), and 25 μg/mL RG1192 (about 180 nM) slightly reduced proliferation compared to control untreated cultures [Fig 2]. Similar results were obtained with three different preparations of RGTA synthesized independently and having similar composition and structure (not shown). All preparations stimulated satellite cell proliferation to about 150% when added at 1 μg/mL. At 25 μg/mL, the proliferation was reduced by about 30% compared to nontreated cultures. Neither of these molecules had a toxic effect or induced any sign of cell suffering at the higher concentration, as measured by the lactate dehydrogenase test assay (not shown). Satellite cell treatments were also performed with heparin [Fig 2]. Heparin slightly stimulated satellite cell proliferation at about 1–5 μg/mL (equivalent to about 300–600 nM). At the highest concentration, it slightly inhibited satellite cell growth. Thus, in this test, heparin appeared about 100 times less efficient than RG1192 in stimulating satellite cell proliferation at similar molar concentrations.

The satellite cell cultures we used were primary cultures in which nonmyogenic cells, principally fibroblasts, were present as contaminant cells. The pronase technique used for satellite cell dissociation has proved to extract a myogenic cell population almost
devoid of contaminating cells. However, in the next experiment, we checked the purity of the population of myoblasts, identified by the presence of desmin, in order to ensure that the presence of RG1192 did not selectively stimulate the proliferation of nonmyogenic cells. We estimated the percentage of desmin-positive cells, which was about 95% at days 5 and 6 of culture in the absence of RG1192, to be 98% and 98.5%, respectively, at days 5 and 6 in RG1192-treated cells. This demonstrated that RG1192 does not selectively alter proliferation of nonmyogenic cells. Rather, if it has an effect, it would be selective relative to desmin-positive myogenic cells.

Relationship between the structure of dextran derivatives and their effect on satellite cell proliferation

In the following experiments, we treated satellite cell cultures with molecules obtained at intermediate steps in the CMBS (RG1192) synthesis (Table I). RG1106 (CMB) or RG1100 (CM) did not stimulate satellite cell proliferation (Table II). For comparison, we also tested the effect of several molecules of commercial origin on proliferation of satellite cells. Dextran sulfate ($M_w = 40$ kDa) was not able to stimulate satellite cell proliferation at 1 µg/mL and was rather toxic at 25 µg/mL, because only 25% of the cells were recorded at day 6 compared to control cultures (Table II). Similar experiments were performed in which DS, HS, or KS from commercial source were tested (Fig. 3). These GAGs have been shown to be synthesized by several myogenic cell lines in vitro. They are involved in the regulation of growth factor activity and indeed, have been shown to stimulate proliferation of myoblasts of cell line 6 when added in the culture medium. Each of these molecules stimulated satellite cell proliferation to a maximum of 130% in the presence of 0.1 µg/mL KS (about 1 nM) or HS (about 2 nM), or 1 µg/mL DS (about 26 nM), assuming that molecular masses of KS, HS, and DS are 109, 50, and 38 kDa, respectively.

RG1192 increased the potentiality of exogenous growth factors in satellite cell proliferation

The following experiment was to determine how RG1192 might stimulate satellite cell proliferation. We hypothesized that these molecules increase the bioavailability of growth factors that control satellite cell proliferation. In cultures that have been starved for 24 h, the addition of serum in the medium allows the cells to enter into new cell cycle and to incorporate ($^3$H)-thymidine. This incorporation was increased in a dose-dependent manner in the presence of RG1192 (Fig. 4). Among growth factors known to control myoblast proliferation, FGF2 is well known to play a pivotal role, and studies concerning the effect of this growth factor on myoblasts are well documented. Satellite cells grown in primary cultures are responsive to the presence of exogenous FGF2. Indeed, in cultures that have been starved for 24 h, the addition of FGF2 in the medium at concentrations from 0.5 ng/mL allows the cells to reenter the cell cycle and incorporate ($^3$H)-thymidine (Fig. 5). In the following experiments, FGF2 was added at a dose of 0.5 ng/mL. RG1192 was added at different concentrations, along with 0.5 ng/mL FGF2, but in the absence
of serum [Fig. 6(A)]. Compared to serum-stimulated cells, cultures treated with RG1192 and FGF2 showed important stimulation of (3H)-thymidine incorporation (about 60% of the maximum incorporation scored in the presence of serum). The response depended on the concentration of RG1192, the optimal concentration being 1–5 \( \mu \text{g/mL} \). The maximum level of incorporation in cells treated with 0.5 ng/mL FGF2 and RG1192 was similar to the level reached in the presence of 5 ng/mL FGF2 used alone. The same results were obtained in the presence of sets of RGTA having equivalent structure (not shown). Similarly, heparin was also able to act in synergy with the FGF2 effect on proliferation, but with less efficiency than RG1192 [Fig 6(B)]. Thus, at comparable molarity, RG1192 was more efficient than heparin in potentiating FGF2 added extracellularly to trigger cells to reinitiate a cell cycle.

Figure 2. Effect of RG1192 or heparin on growth of satellite cells. Primary cultures of satellite cells were treated just after plating with RG1192 or heparin at the specified concentrations. Cell number per plate was determined at days 4, 5, and 6 with a Coulter Counter as described in Methods (Mean ± SE of 4 independent cultures). \( * * * = p < 0.001, * * = p < 0.01, \) and \(* = p < 0.1 \) compared to control.

RG1192 could increase the potentiality of the FGF produced by satellite cells and thus trigger proliferation even in the absence of added growth factors in the medium. This was indeed the case, because RG1192 alone was able to trigger satellite cells to reinitiate a cell cycle in the absence of added growth factor in the medium [Fig. 7(A)]. The stimulation of DNA synthesis was about 25% of that observed in the presence of serum. The effect observed was possibly partially due to FGF2, because DNA synthesis was abolished in the presence of FGF2 neutralizing antibodies [Fig. 7(B)].

SF-HGF, an HBGF, has been known to be expressed in growing and regenerating skeletal muscles. \(^{20,23,50}\) This growth factor has been shown to activate quiescent satellite cells and to drive them into the cell cycle. \(^{50}\) In our experimental conditions, SF-HGF was also able to stimulate (3H)-thymidine incorporation in serum-deprived satellite cells [Fig. 7(C)]. The observed effect of RG1192 on DNA synthesis stimulation was also partially abolished by SF-HGF antibody, suggesting that this growth factor could also be potentiated by RG1192 [Fig. 7(C)].

RG1192 was then shown to increase the potentiality of growth factors, such as FGF or SF-HGF produced by satellite cells, possibly via an autocrine or paracrine mechanism.

RG1192 potentiated growth factors produced by satellite cells

Satellite cells or myogenic cell lines are known to produce FGFs. \(^{10,11,49}\) We therefore tested whether RG1192 partially overcame the effects of chlorate-induced hyposulfonation on cellular growth.

We further examined the possibility that RG1192 could substitute for cellular sulfated GAG in order to stimulate cellular proliferation induced by growth factors. We treated satellite cell cultures with chlorate (30 \( \mu \text{M} \)). This treatment has been shown to suppress sulfate groups on HSPG in myoblasts. \(^{51}\) RG1192, or heparin, was added to the medium after treating cells with chlorate, and cell growth was measured after 48 h (Fig. 8). As shown, RG1192, or heparin, was not able to raise the growth of satellite cells over the basal level of chlorate-treated cultures. This means that these substances alone could not overcome the inhibitory effect of chlorate on cell proliferation by potentiating the effect of growth factors produced endogenously. Interestingly, in the presence of exogenous FGF2, both RG1192 and heparin were able to restore partially cellular growth (Fig. 8).

DISCUSSION

Our results indicated that the dextran derivative RG1192, with a CMBS structure, has the ability to stimulate satellite cell proliferation at about 1 \( \mu \text{g/mL} \) (i.e., about 7 nM). The presence of sulfate appeared
important, because molecules devoid of sulfate (RG1106) had no effect on satellite cell proliferation. Conversely, carboxyl groups were also important, because dextran sulfate was inefficient. Heparin also displayed a stimulating effect on satellite cell proliferation when used at 10 μg/mL (about 600 nM). Thus, RG1192 appeared much more efficient than heparin in stimulating satellite cell growth.

Growth of satellite cells was also shown to be stimulated by natural GAGs such as HS, DS, or KS, which display anionic charges at different rates. HS and DS are known to be part of proteoglycans, such as perlecans, syndecans, or glypicans, known to participate in the regulation of myoblast growth.52–56 KS, in particular, is associated with decorin, which also plays a role in myoblast growth and differentiation by participating in collagen organization.57 Interestingly, RG1192 was as efficient as these GAGs in stimulating satellite cell proliferation. Taken together, these results support the idea that RG1192 mimics natural GAG moiety of proteoglycans in regulating satellite cell growth.

RG1192 also stimulated the proliferation of the myogenic cell lines C2.7 and Sol8, both derived from satellite cells from mice (unpublished data). Other cells have been tested in the presence of RGTAs, such as human intestinal smooth muscle cells58 or pig aortic smooth muscle cells.59,60 RGTAs have different effects on cellular behavior depending on their structure and rate of sulfonation, the cell type, and the growth factors challenged by the presence of the GAG mimetics. Skeletal muscle cells proliferate as a consequence of interactions of growth factor(s) with low-affinity receptors composed of GAGs and/or their high-affinity

### Table II

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 μg/mL</th>
<th>10 μg/mL</th>
<th>25 μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG1192</td>
<td>157 ± 8***</td>
<td>115 ± 9 NS</td>
<td>65 ± 9***</td>
</tr>
<tr>
<td>RG1106</td>
<td>101 ± 12 NS</td>
<td>112 ± 4 NS</td>
<td>105 ± 6 NS</td>
</tr>
<tr>
<td>RG1100</td>
<td>98 ± 7 NS</td>
<td>ND</td>
<td>95 ± 8 NS</td>
</tr>
<tr>
<td>Heparin</td>
<td>122 ± 10*</td>
<td>120 ± 7*</td>
<td>85 ± 5*</td>
</tr>
<tr>
<td>Dextran sulfate</td>
<td>98 ± 7 NS</td>
<td>ND</td>
<td>125 ± 10*</td>
</tr>
</tbody>
</table>

Satellite cells isolated from 2-month-old rats were grown in primary cultures in the presence of Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% fetal calf serum (FCS) and 10% horse serum. They were treated at day 0 with the indicated doses of the different substances listed above. At day 6, the cells were counted. Each value represents mean ± SE of at least 4 cultures (ND = not done).

Statistical analysis of the values compared to nontreated cells: NS = not significant; *** = p < 0.001; * = p < 0.5.

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Figure 3. Effect of dermatan sulfate (DS), keratan sulfate (KS), and heparan sulfate (HS) on satellite cell growth. The experiment was conducted as in Figure 2. Data at day 6 of culture are shown. Concentrations of GAGs are expressed in micrograms per milliliter. RG1192 or heparin (Hep., 10 μg/mL) were added in some cultures. C: untreated control cultures. *** = p < 0.001, ** = p < 0.01, and * = p < 0.05 compared to control.

Figure 4. Increase of serum effect on DNA synthesis by RG1192. At day 4 after plating, cultures of satellite cells were starved for 24 h in the presence of 0.1% bovine serum albumin (BSA). Then complete medium (DMEM + 10% FCS + 10% horse serum) was added either alone or in the presence of RG1192 (A) or heparin (B) at the stated doses. Incorporation was measured in the culture as detailed in Methods. Results are mean ± SE of four cultures. ** = p < 0.01 and * = p < 0.05 compared to serum-treated cultures.
Indeed, as we have shown, effects of FGF2 on satellite cell proliferation were synergized by the GAG mimetic RG1192. Recent investigations of surface plasmon resonance on a Biacore system have shown that FGF2 has a strong binding affinity for RG1192 or heparin ($K_d = 21.7 \text{ nM}$ and $10.6 \text{ nM}$, respectively), whereas it has a very low affinity ($K_d$ in the range of millimoles) for other dextran derivatives devoid of benzylamine or sulfate (Barritault, unpublished data). The affinity of FGF2 for RGTA with the CMBS structure correlates with its proliferating effects on satellite cells.

Whatever type of molecule we used, we observed a bell-shaped effect on the stimulation of proliferation. Excesses of GAG mimetics (RG1192), or of natural GAGs or heparin, limited cellular growth. An inhibitory effect of high-concentration heparin on growth of chick embryo myoblasts has also been reported. The excess of molecules of this type, which would compete with low-affinity receptors, would also reduce the possibility of growth factors interacting with high-affinity receptors at the cellular membrane level and eliciting a cellular response. In regenerating muscle, an excess of RGTA injected either directly in the muscle or systemically reduced the efficiency of the treatment. Taken together, these observations contribute to validation of the in vitro model of primary cultures of satellite cells for testing RGTA molecules.

The mechanism by which the RGTA RG1192 triggers satellite cell response is not yet completely understood. Growth factor interaction with GAG moiety of HSPG might take place through different mechanisms. First, GAG might favor association of growth factor with high-affinity receptors and thus increase growth factor efficiency in triggering cellular response. The RGTA-potentiating FGF2 activity toward satellite cells might possibly be achieved through this mechanism. Other possibilities might be evoked that play a role in the stimulation of satellite cell growth by RGTA. It has been shown that heparin, or analogues such as fucoidan, interact directly with the FGF receptor-4 in the absence of FGF. The consequence of this interaction is the transduction of a signal downstream. Interestingly, the FGF receptor-4 has been shown to be expressed by proliferating rat satellite cells in culture and in satellite cells associated with myofibers. Another mechanism might be that growth factor such as FGF2 can also interact with HSPG and stimulate a cellular response even in the absence of a high-affinity receptor for this growth factor. This has been shown using the L6 myoblast cell line devoid of FGF high-affinity receptor. These cells, when treated with HSPG in the presence of FGF, elicit a cellular response that is different from that obtained in the same cell line expressing an FGF receptor. Alternatively, treatment of cells with heparin...
or GAG mimetic could in turn cause the synthesis and accumulation of heparan sulfate, as shown with smooth muscle cells. Moreover, growth factors, including epidermal growth factor (EGF), FGF, platelet derived growth factor-AB, or IGF-1, can alter the synthesis of GAGs such as DS or HS, independent of their effect on cellular proliferation. Such effect has been illustrated with smooth muscle cells. We cannot rule out the possibility that RGTA might modulate the cellular response through an alteration of GAG synthesis. This approach is being currently examined.

Previous studies have shown that RGTA with CMBS structure (RG1192 or molecules with equivalent substitutions) injected in crushed and/or ischemic and denervated muscle highly stimulated muscle repair. This stimulation, among other criteria, was expressed by the presence of more myofiber in treated muscles compared to controls at day 8 after wounding or healing. The ability of muscle tissue to regenerate in response to injury is dependent on the activation and proliferation of satellite cells. A large number of muscle precursor cells favor the formation of fibers, which would increase in size rapidly with the addition of mononucleated myogenic precursor cells. RGTA injected in injured muscle would favor such an increase of muscle precursor cells. Our results, showing that the growth of satellite cells was stimulated by RGTA, fitted with this assertion. In vivo, in regenerating muscle, satellite cells can be either directly or indirectly stimulated to proliferate according to the great variety of molecules, including growth factors, produced at the site of injury. We cannot exclude the fact that cells other than satellite cells, such as fibroblasts

Figure 7. Potentiation of endogeneous growth factor by RG1192. (A) Experiments were conducted as in Figure 6, but exogenous FGF2 was omitted. Values are mean ± SE of 8 independent cultures. (B) Effect of FGF2 antibodies on RGTA-induced DNA synthesis. After 24 h starvation in the presence of 0.25% serum, cultures were treated with FGF2 (1 ng/mL) or anti-FGF2 (40 μg/mL) or with RG1192 either separately or associated as described. (H)-thymidine incorporation into DNA was then measured as in Methods. (C) Similar experiment was performed in the presence of SF-HGF or antibodies raised against SF-HGF. In that case, cells were treated with the NBTII conditioned medium containing the growth factor, diluted by half in DMEM. Results are mean ± SE of four cultures. ** = p < 0.001 compared to basal level in the presence of 0.25% serum.

Figure 8. RG1192 was able to partially overcome the effect of chlorate-induced hyposulfonation. Satellite cell cultures were treated at day 4 with chlorate (30 mM) for 18 h. Medium was then changed to either 10% FCS% + 10% horse serum, or 30 mM chlorate. Other cultures received chlorate (30 mM) and heparin (10 μg/mL) or RG1192 (1 μg/mL) in the presence or absence of FGF2 (5 ng/mL) as detailed. Results are expressed as number of cells per dish compared to the number of cells in the presence of chlorate alone, taken as reference after 48 h of culture (100%). ** = p < 0.01 compared to the level found in chlorate-treated cells.
or hematopoietic cells from bone marrow, might produce growth factors or cytokines that might in turn change the environment of satellite cells. The fact that the RGTA RG1192 stimulates satellite cell proliferation at least partially explains the improvement of muscle regeneration in its presence. Furthermore, recruitment at least partially explains the improvement of muscle change the environment of satellite cells. The fact that reduce growth factors or cytokines that might in turn produce hematopoietic cells from bone marrow, might pro-

References


