Influence of a dextran derivative on myosin heavy chain expression during rat skeletal muscle regeneration

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Abstract

We recently described dextran derivatives (RGTA) which stimulate tissue repair in several in vivo models. One of them, RGTA11, has been shown to accelerate crush-induced regeneration and reinervation of rat EDL and Soleus muscles. In this study we wanted to know if RGTA11 alters the pattern of myosin heavy chain expression during regeneration. In both EDL and Soleus muscles, RGTA11, injected at the moment of the crush, was found to accelerate the shift from neonatal to adult myosin heavy chain isoforms within 2 weeks. The proportion of slow fibers increased considerably, especially in the Soleus where RGTA11 induced a precocious and permanent expression of slow myosin isoform, thus confirming that a more efficient innervation had occurred in the presence of RGTA11. These results illustrate the interesting potential pharmacological use of such dextran derivatives in neuromuscular disease.

Keywords: Slow and fast muscle; Regeneration; Reinervatior; Dextran derivative; Myosin heavy chain

Skeletal muscle is able to successfully regenerate after injury. Using a model of crush-induced muscle regeneration in rats, we found that the Soleus displayed fibrosis at day 16 after crushing, which finally resulted in the degeneration of the muscle after 30 days [2]. This Soleus degeneration, observed also in other models of regeneration [5,12], probably results from a defect in the process of reinervation following crush [1,5]. In contrast, the regeneration of the fast muscle, Extensor digitorum longus (EDL), was completed 2 months after injury [2]. Recently, we have described several dextran derivatives, obtained by controlled successive substitutions of carboxymethyl, benzylamide and sulfonate groups on glucose residues. In vivo, one of these dextran derivatives, namely RGTA11, has been shown to accelerate skin and bone repair [9]. It has also been shown to stimulate muscle regeneration [7] and reinervation [11] after crushing and denervation. Since innervation is one of the factors known to be involved in the regulation of myosin heavy chain (MyHC) expression [3,14], the aim of the present study was to analyze the effect of RGTA11 on the expression of MyHC in the regenerating Soleus and EDL muscles.

Adult Wistar rats were used in accordance with C.E.E. recommendations for laboratory animal care. Rats were anesthetized with ether, then after sectioning the motor nerve, either the EDL or the Soleus muscles were crushed with forceps from tendon to tendon as previously described [2]. The crushed muscles were injected with 200 µl of PBS containing 10 µg of RGTA11. As controls, crushed muscles were either not injected or were injected with the same volume of PBS. The contralateral non-crushed muscle was referred to as the intact muscle. After 7 and 16 days of regeneration, the muscles were removed from ether-anesthetized animals and directly frozen in liquid nitrogen for further biochemical analyses, or frozen in deep-cooled isopentane for immunocytochemical studies. Analysis of native myosin in muscle extracts by pyrophosphate gel electrophoresis was performed as in [6]. Slow MyHC was detected on 5 µm transverse sections with anti-MyHC antibodies (NovoCastra, France) revealed by the peroxidase-antiperoxidase technique (ABC Vecta Kit). The ratio of slow MyHC-positive fibers to total fibers was determined in each section.
Fig. 1. Transverse sections of intact and regenerating muscles after 16 days of regeneration. (a-c, f-h) Histological aspects after Gomori staining; (d,e,i,j) immunohistochemical staining of slow myosin heavy chain (MyHC) of EDL and soleus muscles respectively. (a) Intact EDL; (b,d) regenerated EDL without treatment; (e,c) regenerated EDL with RGTA11; (f) Intact Soleus; (g,i) regenerated Soleus without treatment; (h,j) regenerated Soleus with RGTA11. Bar = 150 μm.
Fig. 2. Histogram of the percentage of slow myosin heavy chain positive fibers in transverse sections of intact muscles and at day 16 of regeneration, with (+) or without (−) RGTA11. Each point represents the mean ± SD of counts performed on three muscles from three animals.

Muscles which regenerated in the presence of RGTA11 (Fig. 1c,h) displayed a histological structure very similar to that found in intact muscles at day 16 (Fig. 1a,f). In the absence of RGTA11, fibers were smaller in both muscles (Fig. 1b,g). In addition, fibrosis was obvious in the Soleus in the absence of RGTA11 (Fig. 1g). Intact Soleus and EDL muscles contained approximately 92% and 11% of slow fibers, respectively. Crushed control muscles which had regenerated for 16 days showed 6.5% slow fibers in the Soleus and 3.5% in the EDL (Fig. 2). In the RGTA11-treated EDL, this proportion was close to that found in intact muscle. In the Soleus, RGTA11 induced a four-fold increase in the number of slow fibers that reached 27%. Interestingly, slow fibers tended to be in small clusters at the periphery of the muscles. This fiber type grouping was more manifest in the Soleus than in the EDL (Fig. 1) and was enlarged by RGTA11 treatment, especially in the Soleus. The intact EDL muscle was characterized by the expression of three fast isoforms resolved by native gel electrophoresis (Fig. 3a). At day 7 of regeneration (Fig. 3b), three faster migrating bands which corresponded to the neonatal myosin isoforms could be distinguished. These isoforms were progressively replaced by the adult fast isoforms which became the predominant species at day 16 (Fig. 3d). In the intact Soleus, only one slow isoform could be detected (Fig. 3a). At day 7 of regeneration (Fig. 3b), the muscle expressed a mixture of neonatal, fast, and slow isoforms. However, in the absence of RGTA11 the expression of this slow isoform was not stable, since at day 16 it had almost disappeared and the adult fast isoforms were now predominant. RGTA11 treatment induced two effects on these patterns of expression: firstly, it stimulated muscle maturation in both EDL and Soleus muscles (Fig. 3d versus 3e), since at day 16 of regeneration, the adult MyHC isoforms were predominant; secondly, in the Soleus, RGTA11 increased expression of the adult slow MyHC isoform (Fig. 3e) which was only detected as a faint band in the non-injected control muscle (Fig. 3d).

Taken together, RGTA11 was found to induce several major modifications in the pattern of MyHC isoform expression in regenerating muscle: (i) it accelerated the shift from the neonatal to the adult MyHC isoforms in both EDL and Soleus muscles; (ii) it induced a precocious and permanent expression of slow myosin isoform in the Soleus, which was correlated with an increase in the number of slow fibers in this muscle. These results are probably the consequence of a more efficient reinnervation of the regenerating muscle in the presence of RGTA11 as demonstrated by our previous morphological and biochemical analysis [1]. Indeed, in several models, reinnervation was shown not only to be correlated with an increase in the expression of slow myosin, but also with the replacement of the neonatal by the adult fast myosin isoforms [3,4,14]. In addition, we have previously shown that in RGTA11-treated crushed Soleus muscles, the motor end plates differentiated more rapidly than in uninjected controls and were stable and functional [1]. Reinnervation occurs preferentially at the surface of the muscle and involves a certain degree of axonal sprouting. Grouping of the slow

Fig. 3. Electrophoresis under non-dissociating conditions of myosin heavy chain isoforms in the rat EDL and Soleus muscles. (a) Intact muscle; (b) regenerates muscle at day 7 without treatment; (c) regenerates muscle at day 7 injected with RGTA11; (d) regenerates muscle at day 16 without treatment; (e) regenerates muscle at day 16 injected with RGTA11; +, regenerates with RGTA11; −, regenerates without RGTA11; S, slow; F, fast; N, neonatal.
fibers, as shown here, and their localization at the periphery of the muscle, are strong arguments for a precocious reinnervation induced by RGTA11. Our present results, showing that RGTA11 altered the MyHC expression, illustrated some of the interesting properties of this substance. (1) RGTA11 has been proved to act as an antiprotease, particularly of the plasmin type [10]. Therefore, in the presence of RGTA11, the remaining muscular basal lamina containing all the synaptic information would be less damaged and would be able to receive the incoming innervation more rapidly. (2) In addition, RGTA11 has been shown to protect heparin-binding growth factors such as FGFs as demonstrated in vitro [13]. FGFs and other heparin-binding growth factors have been shown to be associated with axon sprouting [8] and synaptogenesis [11]. RGTA11 might therefore facilitate reinnervation, presumably by increasing bioavailability of these growth factors in vivo. These results illustrated the potential pharmacological use that such dextran derivatives could offer in treating neuromuscular disease.

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