Transforming Growth Factor-β2 in Muscle Fibers with Rimmed Vacuoles

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Abstract

Transforming growth factor-β2 (TGF-β2) is associated with tau-containing neurofibrillar tangles in Alzheimer's disease. We report here that the expressions of tau and TGF-β2 are highly correlated in the vacuolated muscle fibres in human and rat myopathies. This implicates TGF-β2 as a regulator of tau in muscle and neurons.

Key words: chloroquine, rimmed vacuole, skeletal muscle, tau, transforming growth factor-β2.

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The formation of neurofibrillar tangles is a characteristic feature of Alzheimer's disease. Neurofibrillar tangles are mainly composed of abnormally phosphorylated tau. Tau is associated with, and promotes the stability of, microtubules [2]. Tau was initially thought to be uniquely expressed by neurones, but more recent work indicates that it is produced by a variety tissues, including skeletal muscles with various pathologies. For example, tau is localised to the rimmed vacuoles present in the muscle fibres of patients with either inclusion body myositis (IBM) [1] or distal myopathy with rimmed vacuole formation (DMRV) [10] and rats with a chloroquine-induced myopathy [9]. Furthermore, the muscles of IBM patients contain phosphorylated-tau-positive twisted tubulofilaments which are similar to the paired helical filaments present in the neurofibrillar tangles of Alzheimer's neurones.

The transforming growth factor-betas (TGF-β) are cytokines which have been linked to Alzheimer's disease. TGF-β2 is expressed by many neurones and is upregulated in the large tangle-bearing neurones in Alzheimer's disease [3, 12]. Altered expression of TGF-βs in the neighbouring glia also occurs, although it is uncertain whether the glia are exclusively producing TGF-β2 or all three of the mammalian TGF-β isoforms [3, 12]. The potential importance of the TGF-βs in Alzheimer's disease is underlined by the recent demonstration that transgenic mice with elevated TGF-β1 expression in their glia develop Alzheimer-like plaques [13].

In this paper, we have used immunohistochemistry and semi-quantitative RT-PCR to examine whether skeletal muscles with abnormal tau also have altered expression of TGF-β2.

Material and Methods

Adult Wistar rats were used in this study. All procedures were approved by the University of Otago's Committee on Ethics in the Care and Use of Laboratory Animals. Three rats were treated with chloroquine for 8 weeks, as previously described [9]. The soleus is particularly affected by chloroquine and was therefore dissected from the treated rats and compared with the soleus from 6 untreated rats. Muscle biopsies from 10 patients with DMRV, 3 with IBM and 2 controls were also examined. These biopsies were used in our previous study of tau expression in vacuolated fibers [9].

The soleus and muscle biopsies used for immunohistochemical studies were frozen in melting isopentane. Transverse 7-µm-thick sections were cut in a cryostat and fixed in either periodate-lysine-paraformaldehyde at 4°C for 10 minutes or in 4% paraformaldehyde in 0.1M sodium phosphate, pH 7.2 at 4°C for 30 minutes. The former sections were incubated in a rabbit anti-human tau antibody [5], diluted 1:200, whereas the latter were incubated in 1.0 µg/ml of rabbit anti-TGF-ß2 antibody (Santa Cruz Biotech.). After incubation at 4°C overnight, the immunoreactivity was developed as previously described [8]. Controls sections were incubated with a similar concentration of non-immune rabbit IgG. The TGF-β2 immunoreactivity was abolished by prior incubation of the antibody with the antigenic peptide.

Total RNA was isolated from frozen soleus with TRIzol reagent (GIBCO-BRL) and cDNAs were generated using a recombinant M-MLV reverse transcriptase (SuperScript II RNase H- reverse transcriptase, GIBCO-BRL) according to the manufacturer's instructions. The cDNAs were amplified using the primers as follows; TGF-β2 [6]: 5'-TCCTACAGACTGGAGTCACAACAG-3' and 5'-ATCATATTGGAAAGCTGTTCGATC-3' and GAPDH [4]: 5'-TTCACACCCATCACAAAG-3' and 5'-CTTCATTGACCTCAACTA-3'. PCRs for TGF-β2 and GAPDH were performed for 24 and 18 cycles, respectively. The products were size-fractionated by electrophoresis through 1.5% agarose gels, transferred to nylon membranes and hybridised with ^{32}P-labelled oligonucleotides as follows; TGF-β2: 5'-TCCTACAGACTGGAGTCACAACAG-3' and GAPDH: 5'-ACTCAGCACCAGCATCACCCCATTTGA-3'. The images of hybridisation signals were captured and quantified by Phosphoimager (Fujifilm) using the analytical software supplied by the manufacturer.

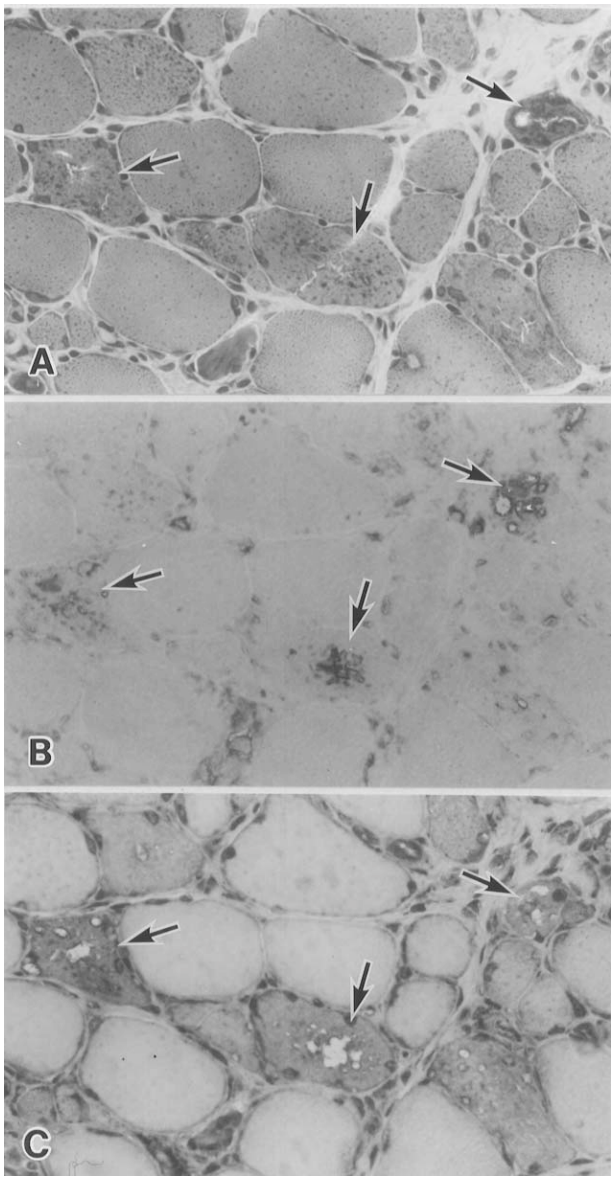

Figure 1. Vacuolated fibres from the soleus of a rat with a chloroquine-induced myopathy. Serial sections were stained with either hematoxylin and eosin (A), an anti-human tau antibody (B) or an anti-TGF-β2 antibody (C). The tau-positive vacuolated fibers (arrows, B) were strongly immunoreactive with the anti-TGF-β2 antibody (C). The magnification is x 350.

Results and Discussion

There was a strong, but incomplete, correlation between the expression of tau and TGF-β2. Almost all of the vacuolated fibres in the DMRV and IBM biopsies reacted strongly with both the anti-tau (see also Murakami et al [11]), and anti-TGF-β2 antibodies (Fig. 1). In the soleus of the chloroquine-treated rats, all of the vacuolated fibres had elevated levels of TGF-β2 but a minority of these fibres lacked detectable levels of tau. The biopsies and chloroquine-treated soleus also contained occasional atrophic angular fibres that lacked vacuoles and tau-immunoreactivity but contained elevated levels of TGF-β2. However, such fibres contained vacuoles in more distal or proximal portions of the fibre.

The immunohistochemical observations were verified using semi-quantitative RT-PCR to measure the levels of TGF-β2 mRNA in the chloroquine-treated soleus. The TGF-β2 specific signals were standardised based on the level of a house-keeping gene (GAPDH). In agreement with the immunohistochemistry, the amount TGF-β2 in the soleus of the chloroquine-treated rats was approximately twice that of normal rat soleus.

The strong association between tau and TGF-β2 in Alzheimer's neurones and vacuolated muscle fibres suggest that these two molecules may be interlinked. TGF-β2 is a regulatory molecule and it is thus possible that TGF-β2 may regulate the expression of tau, particularly since the over-expression of TGF-β1 in transgenic mice leads to Alzheimer-like plaques [13]. However, our detection of vacuolated fibres with elevated TGF-β2, but lacking detectable levels of tau, counts against this possibility. Furthermore, we were also unable to detect tau at the neuromuscular junction, which has high levels of TGF-β2. Thus, the expression of TGF-β2 is not sufficient, by itself, to lead the accumulation of tau. TGF-β2 may, however, be important for triggering tangle formation once tau has accumulated.

High levels of TGF-β2 are expressed in developing, regenerating, denervated and segmentally-damaged muscle fibres [7, 8], as well as the vacuolated fibres described here. In all of these examples, the cytoskeleton of the muscle fibre is undergoing rapid change. The suggests that TGF-β2 may be produced by cells to regulate the assembly or repair of their cytoskeletons. If

so, the synthesis of TGF-β2 by vacuolated fibres may be an attempt to counter the disruption produced by the abnormal accumulation of tau.

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