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RESEARCH ARTICLE

Effect of ageing on the myosin heavy chain composition of the human sternocleidomastoid muscle

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ABSTRACT

The myosin heavy chain (MyHC) composition of ageing limb muscles is transformed into a slower phenotype and expresses fast-twitch fibre type atrophy, presumably due to age-related motor unit remodelling and a change in the patterns of physical activity. It is not known if ageing affects the sternocleidomastoid muscle (SCM) in a similar way. The goal of the study was to analyze the MyHC composition and the size of muscle fibres in the ageing SCM by immunohistochemical methods and quantitative analysis and stereology using our own software for morphometry. We hypothesize that with ageing the MyHC composition of SCM transforms similarly as in ageing limb muscles, but the size of the muscle fibres is less effected as in limb muscles. The study was performed on the autopsy samples of the SCM in 12 older males. The results were compared with those published in our previous study on 15 young adult males.

An ageing SCM transforms into a slower MyHC profile: the percentage of slow-twitch fibres is enhanced (numerical proportion 44.6 vs. 31.5%, $P < 0.05$; area proportion 57.2 vs. 38.4%, $P < 0.05$). The share of hybrid 2a/2x fibres is diminished (numerical proportion 14.1 vs. 26.8%, $P < 0.05$), the area proportion of all fast-twitch fibres expressing MyHC-2a and 2x is smaller (50.6 vs. 63.5%, $P < 0.05$), and the area proportion of fibres expressing the fastest myosin isoform MyHC-2x is smaller too (19.0 vs. 34.5%, $P < 0.05$).

The slower phenotype with the preferential reduction of the fibres expressing the fastest MyHC-2x provide circumstantial evidence for: (i) more fast-twitch than slow-twitch motor units being lost; and (ii) reinnervation by the surviving motor units. There appears to be no significant influence on muscle fibre size, which is congruent with relatively unchanged SCM activity during life.

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1. Introduction

The sternocleidomastoid muscle (SCM) is one member of the muscle group which acts on cervical vertebrae and head (Costa et al., 1990); in addition the SCM is co-activated during chewing (Chandu et al., 2005), is an accessory respiratory muscle (Costa et al., 1994) and its impairment has been demonstrated in cervical osteoarthritis (Falla et al., 2003), spasmodic torticollis and cervical dystonia (Lin and Chou, 1997) as well as in long term repetitive exposure of shoulder/neck muscles to monotonous or static work (Zaza, 1998). Due to its versatile functions, most of which do not change much during life make the SCM an interesting muscle for investigation in health and disease. We have previously reported on the myosin heavy chain (MyHC) composition of the SCM of young subjects (Cvetko et al., 2012). The SCM of young subjects is a fast-twitch muscle with a characteristic MyHC composition – a high share of hybrid fibres 2a/2x, and the co-expression of neonatal MyHC with adult isoforms. In contrast to the rule that neonatal MyHC is usually present in smaller sized regenerating and denervated muscle fibres, the neonatal MyHC was expressed in fibres with normal diameters (Cvetko et al., 2012). In ageing limb muscles fast-to-slow fibre type shift occurs (Lexell, 1995; Nikolic et al., 2001; Mosole et al., 2016; Brocca et al., 2017). It is not yet known whether a similar fast-to-slow fibre type shift also occurs in an ageing SCM. The purpose of the study was to analyze fibre type composition of an ageing SCM to determine whether a fibre type shift similar to that in ageing limb muscles also occurs in ageing neck muscle (SCM). The age related loss of motor neurons has also been described in the cervical spinal cord (Mittal and Logmani, 1987; Zhang et al., 1996; Cruz-Sánchez et al., 1998; Yuan et al., 2000), which means that the neuronal basis for age-related fibre loss (Piasecki et al., 2016) could also be expected in an ageing SCM. Depending on the fibre size, an ageing SCM may behave differently to the limb muscles. In ageing limb muscles, atrophy of type 2

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fibres due to decreased physical activity has been observed, while type 1 fibres are much less affected (Lexell and Taylor, 1991; Lexell, 1995). Conversely, since SCM activity is essential for everyday tasks and remains relatively unchanged during life, pronounced disuse atrophy is probably avoided. Therefore, the SCM appears to be an excellent subject for studying the effects of ageing on MyHC composition without the concomitant influence of disuse. We hypothesize that, with ageing, the MyHC composition of SCM transforms similarly to that in ageing limb muscles, but the size of the muscle fibres is less effected than in limb muscles.

2. Materials and methods

2.1. Subjects

The muscle specimens were excised within 24 h post mortem from the superficial part of the middle portion of the SCM muscle (between the origin and the insertion) from 12 presumably healthy ageing males (aged 60–83 years, $\bar{x} \pm SD = 71.2 \pm 9.8$ years) who had suffered a sudden accident. None of the subjects exhibited signs of a functional disorder of the craniovertebral system. On the basis of the histological examination conducted, none of the muscle specimens exhibited signs of muscular disease. The muscle sampling was approved by the National Medical Ethics Committee of the Republic of Slovenia (permission number 36/04/08).

2.2. Immunohistochemical stainings

The muscle specimens were rapidly frozen in liquid nitrogen and stored at −80 °C. The following monoclonal antibodies specific to MyHC isoforms were applied on 10 μm thick serial transverse sections: BA-D5 antibodies immunoreactive with ß/slow MyHC-1 in rats (Schiaffino et al., 1989) and humans (Smerdu et al., 1994) at a dilution of 1:100; A4-74 antibodies (formerly Alexis Biochemicals, now Enzo Life Sciences, New York, USA) immunoreactive to MyHC-2a and MyHC-2x in humans (Smerdu and Soukup, 2008) and dogs (Smerdu et al., 2005) at a dilution of 1:30; 6H1 antibodies (Developmental Studies Hybridoma Bank, Iowa City, USA) immunoreactive to MyHC-2x in humans (Lucas et al., 2000) at a dilution of 1:3000, MyHC-embryonic (MyHC-emb) and MyHC-neonatal (MyHC-neo) antibodies (both former Novocastra laboratories, Now Leica Biosystems, Newcastle upon Tyne, UK) immunoreactive to developmental MyHC isoforms, the former applied at a dilution of 1:20 and the latter applied at a dilution of 1:10. The BAD-5 antibodies were produced in a local laboratory from the corresponding cell lines provided by Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany).

The muscle fibre phenotyping was performed according to the expression of MyHC isoforms on serial sections by indirect immunoperoxidase method, as described previously (Cvetko et al., 2012; Meznaric and Erzen, 2012).

2.3. Image analysis and statistics

Images of 10 μm serial frozen muscle sections stained by monoclonal antibodies specific to MyHC isoforms, as described above, were captured by a Nikon Eclipse 8000 microscope equipped with a Nikon digitalized camera DXM 1200F and computer software for image acquisition (Lucia GF software, version 4.82, Laboratory imaging, Prague, Czech Republic). Each slow-twitch, fast-twitch and intermediate muscle fibre was delineated using the Ellipse (ViDiTo, Kosice, Slovakia) image analysis program.

The software for muscle fibre type classification and analysis (Karen et al., 2009) was used to classify the muscle fibres and estimate their average diameter and numerical proportions. This program was used to analyze an average of 220 fibres per muscle.

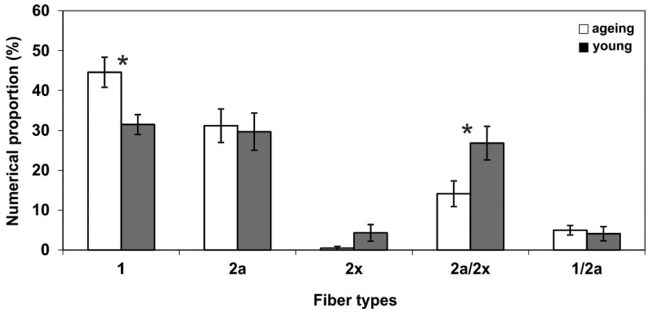

Fig. 1. Numerical proportion (%) of muscle fibres classified on the basis of adult myosin heavy chain (MyHC) isoform expression in the ageing SCM and the SCM of young males. Values are means ± SE. The numerical proportion of slow-twitch fibres was higher in the ageing SCM than in the SCM of young males and the numerical proportion of fast-twitch fibres co-expressing MyHC-2a and 2x was lower (*$P < 0.05$).

The fibres were classified into pure fibres (type 1, 2a, 2x), expressing either MyHC-1, -2a or -2x, hybrid fibres co-expressing two or three adult MyHC isoforms (type 2a/2x, 1/2a, 1/2x and 1/2a/2x) and hybrid fibres co-expressing adult isoforms with the MyHC-neonatal.

Moreover, in approximately 500–600 muscle fibres per muscle that were analyzed from three sample areas, the area fractions of the following were estimated stereologically, applying the point grid plug-in the Ellipse program (ViDiTo, Kosice, Slovakia): (i) slow fibres expressing the MyHC-1 isoform; (ii) all fast-twitch fibres expressing the MyHC-2a and MyHC-2x isoforms; and (iii) the subpopulation of fast-twitch fibres expressing MyHC-2x. The percentage of the MyHC-emb and MyHC-neo positive fibres was determined by counting the number of positive fibres per 1000 muscle fibres.

Statistical analyses were performed using version 5.0 of the SYSTAT statistical package for Windows to calculate the mean values and standard errors for the following: (i) the fibre diameters and numerical proportions for all fibre types and (ii) area proportions of fibres expressing particular adult MYHC isoforms ((i) slow fibres expressing MyHC-1; (ii) all fast fibres expressing MyHC-2a and MyHC-2x isoforms; and (iii) fast fibres expressing only MyHC-2x isoform). An independent samples t-test was used to test the differences between the ageing SCM and the SCM of the young adult males. The results were compared with the data of the 15 young adult males from the previous study on the SCM (Cvetko et al., 2012).

3. Results

3.1. Numerical proportions of fibres expressing adult MyHC isoforms

Five major fibre types (1, 2a, 2x, 2a/2x and 1/2a) were identified: in the ageing SCM as well as in the SCM of young adults, but the proportion of type 1 and 2a/2x differed significantly (Fig. 1). The ageing SCM muscle contained a significantly higher proportion of slow-twitch – type 1 fibres (44.6 vs. 31.5%) and a significantly lower proportion of 2a/2x fibres (14.1 vs. 26.8%). The proportion of type 2a fibres and 1/2a fibres was nearly the same in both groups. The proportion of 2x fibres was smaller in the ageing SCM, but the difference was not statistically significant. Overall, the ageing SCM contained nearly equal numerical proportions of slow- and fast-twitch fibres (44.6 vs. 45.8%), while the SCM of young males was a fast-twitch muscle, of which approximately one-third was slow-twitch fibres and two-thirds were fast-twitch fibres (31.5 vs. 60.8%). In the ageing SCM, similar to the SCM of young males, the MyHC-2x

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