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Age-related changes in the function and structure of the peripheral sensory pathway in mice

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ABSTRACT

This study is aimed at describing the changes occurring in the entire peripheral nervous system sensory pathway along a 2-year observation period in a cohort of C57BL/6 mice. The neurophysiological studies evidenced significant differences in the selected time points corresponding to childhood, young adulthood, adulthood, and aging (i.e., 1, 7, 15, and 25 months of age), with a parabolic course as function of time. The pathological assessment allowed to demonstrate signs of age-related changes since the age of 7 months, with a remarkable increase in both peripheral nerves and dorsal root ganglia at the subsequent time points. These changes were mainly in the myelin sheaths, as also confirmed by the Rotating-Polarization Coherent-Anti-stokes-Raman-scattering microscopy analysis. Evident changes were also present at the morphometric analysis performed on the peripheral nerves, dorsal root ganglia neurons, and skin biopsies. This extensive, multimodal characterization of the peripheral nervous system changes in aging provides the background for future mechanistic studies allowing the selection of the most appropriate time points and readouts according to the investigation aims.

1. Introduction

According to the United Nations (World Population Prospect, 2015 revision, available at http://esa.un.org/unpd/wpp/publications), worldwide life expectancy at birth is projected to rise from 70 years in 2010–2015 to 77 years in 2045–2050, and to 83 years in 2095–2100. This remarkable demographic trend, associated with more careful medical assessment, is accompanied by a parallel increase in the occurrence of diseases typically associated with aging. In the field of neurological disorders, attention has been focused mostly on central nervous system neurodegenerative diseases (e.g., Parkinson's and Alzheimer's disease) in view of their frequency and their major, clearly perceived impact on the daily life activities and autonomy of the affected persons. However, also age-related changes occurring in the sensory component of the peripheral nervous system (PNS) are frequent and may severely compromise the conditions of aged persons. The overall prevalence of peripheral neuropathies is around 2% in the general population, but it raises up to 15% in people older than the age of 40 (Bharucha et al., 1991; Savettieri et al., 1993). Ad hoc epidemiologic studies suggested that the prevalence of peripheral neuropathies steadily increases more in subjects older than 65 years (Beghi and Monticelli, 1998; Monticelli and Beghi, 1993), but patients were not stratified for the presence of concomitant diseases typically occurring with aging and which are potentially able to impair PNS functioning (e.g., diabetes, vitamin deficiency, renal insufficiency, toxics exposure; Martyn and Hughes, 1997).

A few epidemiologic studies confirm that peripheral neuropathies not only steadily increase in frequency but also have worse progression in subject older than 65 years (Beghi and Monticelli, 1998; Gregg et al., 2004; Leblhuber et al., 2011). However, their frequency in elderly persons is probably still underestimated, and their importance in one of the most

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vulnerable and frail category of population is not yet properly admitted.

Clinical, neurophysiological, and pathological changes affecting particularly the sensory component of the PNS functioning are well established in elderly persons, with impaired vibration perception more evident in the distal parts of the limbs, reduced amplitude of nerve potentials, and decreased density of nerve fibers in sural nerve as well as in skin biopsies (Gregg et al., 2004; Mold et al., 2004; Ward et al., 2015). Impaired function of the sensory PNS considerably contributes to other morbidities (Richardson, 2002) and results in pain, foot deformities, amputations, and skin ulcerations as well as in increased risk of falls (Leblhuber et al., 2011; Ward et al., 2014). Motor impairment strictly dependent on peripheral nerve alterations is, by contrast, less frequent, and the evident age-related reduction in strength and motor performances is generally due to a multifactorial sum of events, including musculoskeletal changes (Manini and Clark, 2012). Moreover, sensory function decline has been recognized to be significantly associated with decline in strength in older adults even in the presence of normal motor nerve functioning (Ward et al., 2015).

Although most acquired peripheral neuropathies occur in elderly people in the course of systemic diseases or are caused by toxics exposure or trauma, in the absence of known causes of peripheral neuropathy, it can be assumed that progressive nerve degeneration is per se a feature of aging, independently from other concurrent medical conditions. To support this hypothesis, functional, morphologic, and biochemical changes have been described in the PNS of aged subjects as well as in animal models (Ceballos et al., 1999; Deshpande et al., 2008; Fujimaki et al., 2009; Jeronimo et al., 2008; Melcangi et al., 2003; Shen et al., 2011; Verdú et al., 1996, 2000). Among the possible mechanisms responsible for age-related PNS changes, poor nerve circulation, reduced regeneration from subclinical damage, decreased physiologic turnover of peripheral nerve components, and increased susceptibility to local nerve entrapment have been suggested (Ceballos et al., 1999; Suzuki, 2013).

To investigate on PNS aging, a few studies have been performed in rodents (Ceballos et al., 1999; Jeronimo et al., 2008; Melcangi et al., 2003; Shen et al., 2011; van Nes et al., 2008; Verdú et al., 1996, 2000). However, controversies still exist about the rate of nerve degeneration during the lifetime of the animals, and about possible differences existing among nerves and animal species (Schmelzer and Low, 1987). Moreover, despite the knowledge that pathological changes are not uniformly distributed in the PNS (Ceballos et al., 1999), and that clinical impairment is more severe in the longer leg nerves than in the shorter ones innervating the arms (Leblhuber et al., 2011), no studies evaluated in detail the entire sensory pathway, from the distalmost part represented by skin nerve fibers up to the dorsal root ganglia (DRG).

The aim of this study is to define, using morphological/morphometric and neurophysiological approaches, the pattern of nervous system changes occurring during aging in the entire peripheral sensory pathway from the skin endings to the DRG neurons, in the same cohort of C57BL/6 mice examined longitudinally at different time points over a period of 2 years. Moreover, sampling 2 different nerves, 1 more distal (caudal), and 1 more proximal (sciatic), length-dependent features of normal aging will be compared.

2. Materials and methods

2.1. Animal husbandry

Sixty-six female C57BL/6 mice (Harlan Italy, Corezzana, Italy) aged 4 weeks at the beginning of the study were used. The care and husbandry of animals were in conformity with the institutional guidelines in compliance with national (D. L.vo 26/2014, Gazzetta Ufficiale della Repubblica Italiana, n.61, March 14th 2014) and international laws and policies (European Union directive 2010/63/UE; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996). The study plan was approved by the Ethics Committee of the University of Milan Bicocca (n. 0035125/12). Animals were housed in a limited access animal facility where room temperature and relative humidity were set at 20 °C ± 2 °C and 55% ± 10%, respectively. Artificial lighting provided a 12 hours light/12 hours dark (7 AM–7 PM) cycle. The general condition of the animals was assessed daily, body weight changes were evaluated monthly, whereas caudal and digital nerve conduction studies were performed every 2 months in all animals for a period of 2 years from the study start. Every 2 months, 3 randomly selected mice were sacrificed under isoflurane deep general anesthesia and the isolated sciatic nerve as well as ventral caudal nerve, L4-L5 DRG, and skin samples were collected and processed for morphological and morphometric analysis.

2.2. Hematology and blood chemistry

At 17, 21 months of age, and at the end of the period of observation (25 months of age), whole blood was obtained from all sacrificed animals through caval vein puncture, and serum was obtained by the centrifugation of clotted blood at 2.500 g for 15 minutes at 4 °C and used for determination of the levels of urea, creatinine, aspartate aminotransferase, and alanine aminotransferase as markers of kidney and liver function with an automatic MIRA PLUS system (Horiba ABX Diagnostic, Montpellier, France; Cavaletti et al., 2013).

2.3. Neurophysiology

The neurophysiological evaluations were performed in a temperature-controlled room, under isoflurane anesthesia, monitoring animal vital signs and body temperature. The nerve conduction velocity (NCV) and the amplitude of the sensory potential (SAP) were measured in the caudal and digital nerves using an electromyography apparatus (Myto2 ABN Neuro, Firenze, Italy). Caudal nerve neurophysiological assessments were performed by placing a couple of recording needle electrodes at the base of the tail and a couple of stimulating needle electrodes 3.5 cm distally to the recording points. Similarly, the digital nerve assessments were performed by placing the positive recording electrode in the thigh, the negative recording electrode close to the ankle bone, and the positive and negative stimulating electrodes close to the fourth toe near the digital nerve and under the paw, respectively. The intensity, duration, and frequency of stimulation were set to obtain optimal results, as previously described in detail (Carozzi et al., 2010, 2015; Meregalli et al., 2015; Renn et al., 2011).

2.4. Pathological evaluation of peripheral nerves and DRG

Every 2 months, 3 animals were sacrificed under deep anesthesia. Sciatic and ventral caudal nerves were isolated at the same anatomical point (mid-thigh or 1 cm from the base of the tail, respectively) and dissected out without stretching. The specimens were fixed by immersion in 3% glutaraldehyde in 0.12-M phosphate buffer solution, postfixed in OsO_4, epoxy resin embedded, and used for light and electron microscopic observations and for morphometric analysis. L4-L5 DRG were carefully isolated and collected in 3 animals. The specimens were fixed by immersion in 4% paraformaldehyde and 2% glutaraldehyde in 0.12-M phosphate buffer solution, postfixed in OsO_4, epoxy resin embedded, and used for light and electron microscopic observations and for