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The effect of aging on the density of the sensory nerve fiber innervation of bone and acute skeletal pain

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Abstract

As humans age there is a decline in most sensory systems including vision, hearing, taste, smell, and tactile acuity. In contrast, the frequency and severity of musculoskeletal pain generally increases with age. To determine whether the density of sensory nerve fibers that transduce skeletal pain changes with age, calcitonin gene related peptide (CGRP) and neurofilament 200 kDa (NF200) sensory nerve fibers that innervate the femur were examined in the femurs of young (4-month-old), middle-aged (13-month-old) and old (36-month-old) male F344/BNF1 rats. Whereas the bone quality showed a significant age-related decline, the density of $CGRP^+$ and $NF200^+$ nerve fibers that innervate the bone remained remarkably unchanged as did the severity of acute skeletal fracture pain. Thus, while bone mass, quality, and strength undergo a significant decline with age, the density of sensory nerve fibers that transduce noxious stimuli remain largely intact. These data may in part explain why musculoskeletal pain increases with age.

Keywords: Periosteum; Orthopedic; Elderly; Fracture healing; Analgesia

1. Introduction

Chronic musculoskeletal pain caused by conditions such as osteoarthritis, fractures, and low back disorders becomes more prevalent with age (Felson et al., 1987; Muraki et al., 2009; Woolf and Pfleger, 2003). These chronic skeletal pains, as well as the more acute musculoskeletal pain that follows moderate to strenuous physical exercise also increases with age (Fell et al., 2008), and all contribute to a loss of mobility, functional status, and quality of life (Brooks, 2006; Dominick et al., 2004; Woolf and Pfleger, 2003). Importantly, as the life expectancy in the developing and developed world is markedly increasing (Lutz et al., 2008), the toll that musculoskeletal pain will exact on individuals and society is also predicted to increase (Brooks, 2006; Woolf and Pfleger, 2003).

A major reason why musculoskeletal pain is such a medical and social burden is that our understanding of the factors that generate and maintain musculoskeletal pain is limited and even less is known about the mechanisms driving skeletal pain in older individuals. Currently, what we do know is that bones in older individuals (>50 years old) are more fragile than bones in younger individuals and less capable of sustaining damage before failure (Burr and Turner, 1999; Burstein et al., 1976; Currey et al., 1996). Equally important is that the ability of bone to absorb energy is significantly reduced with age, so that injury or fracture is much more likely to occur in an old bone than in a young bone upon impact loads of significant magnitude (Burr and Turner, 1999; Zioupos and Currey, 1998).

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While there is an exhaustive literature on how bone deteriorates and becomes more fragile with age (Burr and Turner, 1999; Burstein et al., 1976; Currey et al., 1996; Zioupos and Currey, 1998), we know remarkably little about how the sensory nerve fibers that innervate the skeleton change with age. Currently, there are 3 major hypotheses as to why musculoskeletal pain increases with age. First, with age, the quantity and quality of bone decreases so that significant use and/or loads are more likely to lead to injury of bone tissue. Second, it has been hypothesized that musculoskeletal pain increases with age because the descending inhibition of pain in the spinal cord decreases with age (Edwards et al., 2003; Washington et al., 2000). Lastly, it has also been postulated that the brain's perception of pain is different in the aged versus the young (Cole et al., 2010). An additional untested hypothesis is that unlike many other sensory systems, the density of the musculoskeletal pain sensory system does not undergo significant decline with age. Thus, while the bone itself declines in terms of mass, quality, and strength with age, if the sensory nerve fibers that innervate the bone remain largely intact, one would expect ever-increasing sensitization and activation of these nerve fibers as the bone is loaded and/or undergoes significant stress. Thus, the hypothesis that will be tested here is that while there is an age-related decline in bone progenitor cells, bone quality, bone mass, and fracture healing, there will not be a significant decline in the density of the sensory nerve fibers innervating the bone nor a decline in the severity of acute fracture-induced pain.

2. Methods

2.1. Animals

All procedures were approved by the Institutional Animal Care and Use Committee at the VA Medical Center (Minneapolis, MN, USA) and the University of Arizona (Tucson, AZ, USA) and were in accordance with the National Institutes of Health guidelines for care and use of laboratory animals. All efforts were made to minimize the suffering and number of animals used.

The 25- and 36-month-old, naive male Fischer 344/Brown Norway F1 (F344/BNF1) rats were a generous donation from Dr. Prisca Honore (Abbott Laboratories, Abbott Park, IL, USA). Young (4- and 5-month-old) and middle-aged (13-month-old) F344/BNF1 rats were purchased from Harlan Laboratories (Indianapolis, IN, USA). The F344/BNF1 rat is recommended for age-related studies by the National Institutes on Aging because this hybrid rat lives longer and has a lower rate of pathological conditions than inbred rats (Walker et al., 2006). The rats were housed in conventional facilities with a 12-hour light/dark cycle and given food and water *ad libitum.*

2.2. Bromodeoxyuridine administration

To visualize proliferating cells in the periosteum under normal conditions, rats were placed under isoflurane anesthesia and implanted subcutaneously with 2 miniosmotic pumps (model 1003D, Alzet, Cupertino, CA, USA) containing ~100 μL of 200 mg/mL bromodeoxyuridine (BrdU, Sigma, St. Louis, MO, USA) solution. Rats were sacrificed 7 days post pump implantation and processed for immunohistochemistry (see below).

Because the number of cells labeled with BrdU following parenteral injections may be limited by the very short bioavailability of BrdU (half-life of 8 minutes; Taupin, 2007), studies designed to identify changes in the dividing cells due to age or to other factors have adopted and validated the use of continuous administration of BrdU by using miniosmotic pumps. This type of administration spans several days and allows obtaining consistent and reproducible intensity of BrdU staining (Baldauf and Reymann, 2005; Cao et al., 2007; Soames et al., 1994; Tatematsu et al., 1989). Additionally, a previous study performed in bone showed sustained administration of BrdU via osmotic minipump provided a more substantial signal to noise ratio and reproducible immunostaining of BrdU in the periosteal cells as compared with single and multiple injections (Barou et al., 1997). Preliminary studies in our laboratory confirmed these results. In light of these findings, we administered BrdU by using miniosmotic pumps.

2.3. Euthanasia and microcomputed tomography (μCT) analysis

Naive rats were euthanized with CO_2 and perfused intracardially as previously described (Jimenez-Andrade et al., 2009). The femurs were removed, postfixed for 4 hours in perfusion fixative, and placed in phosphate-buffered saline (PBS) solution at 4 °C. To characterize the age-related changes in mineralized bone microarchitecture, femurs were analyzed with an eXplore Locus SP microcomputed tomography (μCT) system (GE Healthcare, London, Ontario, Canada). This cone beam μCT scanner used a 2300 × 2300 charged couple device detector with current and voltage set at 80 μA and 80 KVp, respectively. Specimens were scanned in 900 views through 360° with a 2100 ms integration time. Scans were then reconstructed at 16 μm^3 resolution using Reconstruction Utility software v.1.0 (GE Healthcare, London, Ontario, Canada).

To visualize overall differences in trabecular and cortical bone, μCT images of preprocessed femurs were rendered using the MicroView analysis software ABA 2.2 (GE Healthcare, London, Ontario, Canada) and assembled using Adobe Photoshop CS and Adobe Illustrator CS4.