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Behavioral signs of pain and functional impairment in a mouse model of osteogenesis imperfecta

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ABSTRACT

Osteogenesis imperfecta (OI) is a congenital disorder caused most often by dominant mutations in the *COL1A1* or *COL1A2* genes that encode the alpha chains of type I collagen. Severe forms of OI are associated with skeletal deformities and frequent fractures. Skeletal pain can occur acutely after fracture, but also arises chronically without preceding fractures. In this study we assessed OI-associated pain in the Col1a1Jrt/+ mouse, a recently developed model of severe dominant OI. Similar to severe OI in humans, this mouse has significant skeletal abnormalities and develops spontaneous fractures, joint dislocations and vertebral deformities. In this model, we investigated behavioral measures of pain and functional impairment. Significant hypersensitivity to mechanical, heat and cold stimuli, assessed by von Frey filaments, radiant heat paw withdrawal and the acetone tests, respectively, were observed in OI compared to control wildtype littermates. OI mice also displayed reduced motor activity in the running wheel and open field assays. Immunocytochemical analysis revealed no changes between OI and WT mice in innervation of the glabrous skin of the hindpaw or in expression of the pain-related neuropeptide calcitonin gene-related protein in sensory neurons. In contrast, increased sensitivity to mechanical and cold stimulation strongly correlated with the extent of skeletal deformities in OI mice. Thus, we demonstrated that the Col1a1Jrt/+ mouse model of severe OI has hypersensitivity to mechanical and thermal stimuli, consistent with a state of chronic pain.

1. Introduction

Osteogenesis imperfecta (OI) or brittle bone disease is associated with bone fragility, bone deformities, short stature and many other skeletal manifestations of widely varying severity. Extra-skeletal abnormalities associated with OI include joint hyperlaxity, muscle weakness, brittle teeth, bluish-gray sclera and hearing defects. The prevalence of OI at birth is about 1 in 10,000 [1]. In the large majority of individuals with OI, the disorder is caused by mutations in genes encoding collagen type I, *COL1A1* and *COL1A2*.

Skeletal pain is a major issue in OI patients [2]. Even mild forms of OI pain can be associated with decreased health-related quality of life [3]. However, scientific data on the topic remain scarce. The most extensive study on pain in OI obtained a 1-week pain diary in 35 children and concluded that both acute and chronic pain were common and interfered with activities of daily life [2]. Thus, even though the importance of pain in OI is universally acknowledged, little is known about the underlying mechanisms and no mechanistic studies on pain in OI animal models have been reported.

Here we used the recently developed *Col1a1*^{Jrt}/+ mouse model of OI to study OI-related pain. *Col1a1*^{Jrt}/+ (OI) mice exhibit small stature, low bone mineral density and fragile bones similar to type IV OI patients [4]. In mice, evoked responses to sensory noxious stimuli (nociception) of different modalities (mechanical, heat and cold) serve as proxy behavioral indices suggestive of increased pain sensitivity [5]. Other behavioral measures of functional impairment that may be secondary to pain include decreased motor activity and limping.

OI and WT mice were tested for behavioral signs of sensory hypersensitivity and functional impairment. The OI mice displayed significant hypersensitivity and reduced motor ability compared to WT mice. There was a significant correlation between some evoked behavioral responses and skeletal deformities in OI mice. In contrast, no differences

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were observed in measures of either cutaneous innervation density or sensory neuron plasticity. This study provides insights into the relationship between bone health and behavioral indices of pain and functional impairment in WT and OI mice.

2. Materials and methods

2.1. Animals

All experiments were approved by the Animal Care Committee at McGill University and conformed to the ethical guidelines of the Canadian Council on Animal Care and the guidelines of the Committee for Research and Ethical Issues of IASP [6]. The *Col1a1*^{Jrt}/+ mice were developed by screening of N-ethyl-N-nitrosourea-induced mutagenesis resulting in T to C transition in the COL1A1 gene leading to an 18 amino acid deletion in the main triple helical domain of Col1a1, as described in [4]. The *Col1a1*^{Jrt}/+ mice were bred on a FVB background. Mice were a gift from Dr. Jane Aubin's laboratory, University of Toronto. The breeding colony was maintained at the Animal Care Facility of the Shriners Hospitals for Children®—Canada. Animals had unrestricted access to food and water, and were on a 12-hour alternating light and dark cycle. A total of 29 (3–6 month old males); 15 WT and 14 *Col1a1*^{Jrt}/+ mice were used in the study.

2.2. Behavioral assessment

Animals were transferred to the Alan Edwards Centre for Research on Pain and habituated for one week prior to testing. Behavioral measurements were taken weekly for 7 consecutive weeks and at week 10. Animals were acclimatized for one hour prior to each testing session.

2.2.1. Behavioral measures of sensitivity to mechanical, heat and cold stimuli

2.2.1.1. Mechanical hypersensitivity. Mechanical hypersensitivity was performed using von Frey filaments (Stoelting Co., Wood Dale, IL). Animals were placed on an elevated wire mesh grid and covered individually with glass compartments. Filaments were applied to the plantar surface of the hind paw to the point of bending. A positive response was defined as a withdrawal from the filament within two seconds. The fifty percent withdrawal threshold in grams was calculated according to the up-and-down method [7]. A decrease in withdrawal threshold corresponds to an increase in mechanical sensitivity.

2.2.1.2. Heat hypersensitivity. Heat hypersensitivity was measured using the IITC Life Science Inc. plantar analgesia meter as previously described [8]. A radiant heat beam was directed to the planter surface of the hind paw and latency to withdrawal was recorded in seconds. A cut off was set to 22.7 s to avoid tissue damage. Decreases in withdrawal latency correspond to increased sensitivity to heat stimuli.

2.2.1.3. Cold hypersensitivity. Cold hypersensitivity was adapted from Choi et al. [9]. Cold hypersensitivity test was performed by placing a drop of acetone (~25 μL) on the planter surface of the hind paw using a syringe loaded needle. Total time spent engaging in acetone-evoked behaviors such as lifting and shaking of the paw was recorded for one minute. Increases in acetone-evoked behaviors are suggestive of increased sensitivity to cold.

2.2.2. Behavioral measures of functional impairment

2.2.2.1. Open field assay The open field assay. Was performed by placing mice individually in a transparent Plexiglas chamber with 40 cm high walls and a square base of 27 × 27 cm. A video camera was placed on the top of the chamber to track the animals' motor activity with ANY-maze software (Stoelting, USA) for 5 min. Total distance traveled was determined.

2.2.2.2. Rearing. Rearing was determined by an observer blind to genotype as the time spent standing on the animals hind limbs in an upright position during the open field assay. Rearing time was recorded in seconds.

2.2.2.3. Limping score. A limping score modified from [10], was given to each mouse by an observer blind to genotype after 5 min of non-forced ambulation in the open field setting. The assigned scale was as follows: 0 = complete lack of limb use, 1 = partial non-use of the limb in locomotor activity, 2 = limping and guarding behavior, 3 = substantial limping, 4 = normal use. A score was given to each limb according to the scale. An average score of all four limbs was given to each animal.

2.2.2.4. Voluntary running wheel test. The voluntary running wheel test was performed by placing an individual mouse in a home cage containing a rotating wheel with sensor, wireless transmitter and receiving software (Med Associates, Inc.). Voluntary running behavior was measured as the number of wheel rotations detected during a one hour test period.

2.3. Radiography

Live digital radiographs (Faxitron® MX-20) were taken at weeks 3, 8 & 11. Mice were anesthetized with an intraperitoneal (i.p.) injection of 0.01 mL/g of ketamine (100 mg/mL), xylazine (20 mg/mL) and acepromazine (10 mg/mL). Animals were scanned in a prone position after taping to the exact location for each scan. Radiographs were analyzed by two independent observers blind to genotype. Skeletal abnormalities including atlanto-occipital joint dislocation, scoliosis-like spine deformity, reduced cervical intervertebral disc space, reduced length of long bones, deformation and fracture of olecranon processes, deformation of coxae, arthritic knees, hindpaw and forepaw bone deformities, loss of transverse processes of caudal vertebrae, hyperplastic callus formation, and osteophytes were recorded for each mouse and a skeletal deformity score was calculated as a total number of all observed abnormalities.

2.4. Tissue processing for micro-computed tomography and immunohistochemistry

Mice were deeply anesthetised and perfused transcardially with vascular rinse (5% 0.2 M phosphate buffer, 0.95% NaCl, 0.026% KCl, 0.05% $NaHCO_3$ in distilled water, 0.1% $NaNO_2$ added at day of perfusion) followed by 50 mL of 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). Femurs were cleaned thoroughly, stored in 70% ethanol at 4°C then scanned by Micro Computed Tomography. Glabrous hindpaw skin was cryoprotected in 30% sucrose in phosphate-buffered saline (PBS) overnight at 4°C, embedded in optimum cutting temperature medium (OCT; Tissue Tek®) and sectioned (40 μm). Upper lumbar dorsal root ganglia (DRG) were dissected, post-fixed in 4% paraformaldehyde in phosphate buffer for one day at 4°C, cryoprotected in 30% sucrose in PBS for 4 days at 4°C, embedded in OCT, sectioned at a thickness of 10 μm and thaw-mounted onto gelatin-coated slides.

2.5. Micro-computed tomography

Right femurs were scanned in PBS using cone beam computed tomography (CT) (Skyscan 1172) at a voxel size of 6 μm. Scan parameters included a 0.45-degree increment angle, 3 frames averaged, an 84-kVp and 118-mA X-ray source with a 0.5-mm Al filter to reduce beam hardening artifacts. Trabecular bone was analyzed in a region starting at 0.5 mm proximal to the distal femoral growth plate (to avoid primary