Bone 103 (2017) 168–176

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

A novel role for dopamine signaling in the pathogenesis of bone loss from the atypical antipsychotic drug risperidone in female mice

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ARTICLE INFO

Article history:
Received 15 February 2017
Revised 30 June 2017
Accepted 5 July 2017
Available online 06 July 2017

Keywords:
Bone
Atypical antipsychotic drug
Risperidone
Hypogonadism
Dopamine
Osteoclast

ABSTRACT

Atypical antipsychotic (AA) drugs, including risperidone (RIS), are used to treat schizophrenia, bipolar disorder, and autism, and are prescribed off-label for other mental health issues. AA drugs are associated with severe metabolic side effects of obesity and type 2 diabetes. Cross-sectional and longitudinal data also show that risperidone causes bone loss and increases fracture risk in both men and women. There are several potential mechanisms of bone loss from RIS. One is hypogonadism due to hyperprolactinemia from dopamine receptor antagonism. However, many patients have normal prolactin levels; moreover we demonstrated that bone loss from RIS in mice can be blocked by inhibition of β-adrenergic receptor activation with propranolol, suggesting the sympathetic nervous system (SNS) plays a pathological role. Further, when, we treated ovariectomized (OVX) and sham operated mice daily for 8 weeks with RIS or vehicle we demonstrated that RIS causes significant trabecular bone loss in both sham operated and OVX mice. RIS directly suppressed osteoblast number in both sham and OVX mice, but increased osteoclast number and surface in OVX mice alone, potentially accounting for the augmented bone loss. Thus, hypogonadism alone cannot explain RIS induced bone loss. In the current study, we show that dopamine and RIS are present in the bone marrow compartment and that RIS can exert its effects directly on bone cells via dopamine receptors. Our findings of both direct and indirect effects of AA drugs on bone are relevant for current and future clinical and translational studies investigating the mechanism of skeletal changes from AA drugs.

1. Introduction

Atypical antipsychotic (AA) drugs are approved to treat schizophrenia and bipolar disorder, and some are approved to treat irritability associated with autism in children. Despite FDA warning labels relating to safety issues in the elderly, over 20% of nursing home residents are prescribed AA drugs [1]. Additionally, many children, adolescents, and adults are prescribed AA drugs for diagnoses with no FDA-approved indication for AA use [2]. Although AA drugs were initially developed to minimize neurological side effects (akathesia, dystonia and Parkinsonism) caused by typical (a.k.a. first generation) antipsychotic drugs, they have significant metabolic side effects. These include obesity, hyperglycemia, hyperphagia, sleep disruption, dyslipidemia, hyperprolactinemia and cardiac arrest [3–6]. Furthermore, clinical data are emerging that bone is another "off-target" site for AA drugs. Fracture risk is elevated in schizophrenic patients who take AAs compared to the general population and AA-induced bone changes may account for a portion of this increased risk [7]. Also, limited studies show that patients treated with risperidone (RIS, one of the first approved AA drugs which continues to be widely used) and other AA drugs have reduced bone mineral density (BMD) and increased fracture risk [8–16]. These data are especially concerning in populations such as the elderly and anorexia nervosa patients where fracture risk is already elevated. With such a large patient population taking AA drugs, it is critical to understand the underlying pharmacology associated with these undesirable health effects. Unfortunately, there are relatively few clinical or basic research studies focused on this problem.

All antipsychotic drugs have complex receptor pharmacology, with high affinity for dopamine, serotonin and other G-protein coupled receptors. However, compared to first-generation antipsychotic drugs that are potent dopamine receptor antagonists, AA drugs are moderate dopamine receptor antagonists, with increased potency at several serotonin receptors [17,18]. Moreover, the pharmacologic mechanisms of the metabolic and endocrine side effects of AA drugs are largely

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http://dx.doi.org/10.1016/j.bone.2017.07.008
8756-3282/

unknown. Although the mechanism for AA-induced bone loss has not been established, many investigators postulate there is a disruption in the hypothalamic-pituitary-gonadal axis [19]. Dopamine action tonically inhibits prolactin secretion from the anterior pituitary. Predictably, then, AA drugs with higher peripheral exposure have been associated with clinical hyperprolactinemia. Hyperprolactinemia can cause hypogonadism, as observed in patients with prolactin-secreting tumors [20]. However, not all patients treated with AA drugs present with hyperprolactinemia and hypogonadism, and there is little evidence supporting a direct link between AA-induced hyperprolactinemia, bone mass and fracture risk in the clinical literature.

In our mouse model of RIS treatment, we previously reported bone loss and uncoupled remodeling, as would be expected from hypogonadism [21]. To determine whether bone loss observed with RIS treatment is a consequence, or independent, of hypogonadism, we treated ovariectomized (OVX) mice with RIS. We hypothesized that if hypogonadism alone was responsible for RIS-mediated bone loss, OVX mice would not lose any additional bone with RIS treatment. Briefly, we found significant trabecular bone loss from RIS in both the SHAM and the OVX mice. This confirms that despite the potential for hyperprolactinemia with our clinically relevant dose of RIS, hyperprolactinemia-induced hypogonadism cannot fully explain RIS-induced bone loss. In this study, we hypothesized there were two additional mechanisms, SNS-mediation and direct inhibition of dopamine signaling in the osteoclast, [21,22].

2. Materials and methods

2.1. Rats and drug treatment

Animal studies were conducted according to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The Institutional Animal Care and Use Committee of the University of New England approved all rat protocols. Eight-week-old male Sprague-Dawley rats (Charles River Laboratories) were treated with vehicle (VEH; methylcellulose), or a clinically relevant dose of RIS (0.03 and 0.3 mg/kg). Plasma was collected at 2, 6, and 24 h post-dose. The choice of rats for prolactin measurements was due to the difficulty with measuring prolactin using commercially-available reagents for mice.

2.2. Risperidone measurement in plasma and marrow

Concentrations of risperidone and its active metabolite, 9-OH risperidone, were measured in plasma collected from rats and mice by reversed phase liquid chromatography/mass spectrometry (LC/MS/MS) as previously described [22]. In order to evaluate systemic exposure of risperidone and other AA medications in vulnerable tissues such as bone, we developed a method for the extraction and detection of risperidone and 9-OH risperidone from bone marrow (BM). Briefly, extraction of the two analytes from 20 μL of BM was accomplished using an acetonitrile protein precipitation procedure, employing paroxetine as an internal standard. Detection and quantitation were achieved by LC/MS/MS. Method details are available in the Supplementary methods section. Pharmacokinetic data was analyzed using Phoenix WinNonlin (Certara USA, Inc., Princeton, NJ).

2.3. Prolactin measurements

Rat plasma prolactin concentrations were quantified by ELISA following the manufacturer's protocol (SPI BIO/Cayman Chemical, Ann Arbor, MI). Samples were performed in duplicate and absorbance was measured at 414 nm using a SpectraMax M5 plate reader (Molecular Devices, Sunnyvale, CA).

2.4. Mice and drug treatment

The Institutional Animal Care and Use Committees of the University of New England and the Maine Medical Center Research Institute approved all mouse protocols. Female SHAM operated and ovariectomized (OVX) C57BL6/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were operated on at 5 weeks of age, and then sent to MMCRI at 7 weeks of age. At 8 weeks of age, mice underwent baseline measurements (DXA, below), were randomized, and initiated either 0.75 mg/kg risperidone (RIS) or vehicle (VEH, 0.1% acetic acid) administration daily by oral gavage for 8 weeks (from 8 to 16 weeks of age). The dose of risperidone was chosen based on dose-ranging pharmacokinetic/pharmacodynamic studies and represents clinically relevant exposure determined by LC/MS/MS in the plasma [22]. This dose causes significant trabecular bone loss without affecting adipose tissue mass in female C57BL/6J mice [22].

2.5. Dual-energy X-ray absorptiometry (DXA)

All mice were measured at baseline (8 weeks) and at the time of sacrifice (16 weeks) for fat-free mass, fat mass, and bone mineral density using the PIXImus dual-energy X-ray densitometer (GE-Lunar, Madison, WI, USA). The PIXImus was calibrated daily with a mouse phantom provided by the manufacturer. Mice were placed ventral side down with each limb and tail positioned away from the body. Full body scans were obtained, and X-ray absorptiometry data were gathered and processed with the manufacturer's supplied software (Lunar PIXImus 2, version 2.1). The head was specifically excluded from all analyses due to concentrated mineral in skull and teeth.

2.6. Serum remodeling markers

Serum P1NP and CTx levels were measured using commercially available kits from IDS (Gaithersburg, MD) according to the manufacturer's instructions. The assay sensitivities were 0.7 and 2 ng/mL for P1NP and CTx, respectively. The intra-assay variations were 6.3 and 6.9%, and the inter-assay variations were 8.5 and 12% respectively, for both assays. All measurements were performed in duplicate.

2.7. Micro-computed tomography (μCT)

Microarchitecture of the distal trabecular bone and midshaft cortical bone of the femur, and of the trabecular bone of the L5 vertebrae, was analyzed by μCT as previously described (resolution 10 μm, VivaCT-40, Scanco Medical AG, Bassersdorf, Switzerland) [21]. Measurements included bone volume/total volume (BV/TV), trabecular number (Tb.N.), trabecular thickness (Tb.Th.), trabecular separation (Tb.Sp.), and connectivity density (Conn.D). Scans for the cortical region were measured at the mid-point of each femur, with an isotropic pixel size of 21 μm and slice thickness of 21 μm, and used to calculate the average bone area (BA), total cross-sectional area (TA), bone area/total area (BA/TA), and cortical thickness (Ct.Th.). For cortical porosity measurements we used a fixed threshold of 700 mg HA/cm^3. All scans were analyzed using manufacturer software (Scanco, version 4.05).

2.8. Histology and quantitative histomorphometry

Qualitative histologic analysis and quantitative static and dynamic histomorphometry were performed as described previously [23]. To examine bone-formation rates, calcein label (20 mg/kg) and demeclocycline label (20 mg/kg) were injected i.p. 9 and 2 days prior to sacrifice, respectively. Immediately after sacrifice, tibias for histomorphometry were placed in 70% ethanol and maintained in the dark at 4 °C. Histomorphometric measurements were performed on the secondary spongiosa of the proximal tibia metaphysis using an OsteoMeasure morphometry system (Osteometrics, Atlanta, GA, USA).