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SHORT NOTE

Bisphosphonates and MeCP2 Deficiency: Cellular Studies and Clinical Application in Rett Syndrome

Jay R Shapiro[1] and Mary E Blue[2,3]*

[1]*Department of Medicine, Uniformed Services University of the Health Sciences, USA*

[2]*Hugo W. Moser Research Institute at Kennedy Krieger Inc., USA*

[3]*Department of Neurology and Neuroscience, School of Medicine, Johns Hopkins University, USA*

***Corresponding author:** *Mary E Blue, PhD, Hugo W. Moser Research Institute at Kennedy Krieger Inc.; Department of Neurology and Neuroscience, School of Medicine, Johns Hopkins University, Room 400R, 707 North Broadway, Baltimore, MD 21205, USA, Tel: 1-443-923-2693, Fax: 1-443-923-2695, E-mail: blue@kennedykrieger.org*

Abstract

In this Short Note, we assess what is known about the structural deficits in bone in Rett syndrome (RTT), a rare x-linked neurodevelopmental disorder and in mouse models of RTT and provide a rationale for the treatment of bone fragility observed in this disorder. RTT is caused by mutations in a gene that encodes for methyl CpG protein binding protein 2 (MeCP2), which can act as an inhibitor or activator of gene transcription. MeCP2 is ubiquitously expressed throughout the body. Not surprisingly, MeCP2 deficiency affects multiple organ systems and is associated with changes in cognition, respiratory function, muscle tone, bone development and the autonomic nervous system, with tremors and ataxia, seizures and with stereotypic hand grasping movements. We review the literature concerning bone deficits in RTT and in mouse models of MeCP2 deficiency and the effects of the bisphosphonate zoledronic acid on these bone alterations. We conclude that bisphosphonates offer promise for the treatment of bone abnormalities in RTT.

Keywords

Rett syndrome, Bone, MeCP2, Bisphosphonates, Osteoporosis, Mouse model

Short Note

Rett syndrome (RTT) is a rare X-linked neurodevelopmental disorder primarily affecting females. Phenotypic features include intellectual disability, alterations in respiratory function, muscle tone, bone development and the autonomic nervous system, tremor and ataxia, seizures and stereotypic hand grasping movements [1-4]. RTT is caused by mutations in the gene that encodes methyl-CpG-binding protein-2 (MeCP2) [5], a global inhibitor or activator of DNA transcription [6]. While osteoporosis and scoliosis have long been a well-recognized features of RTT [3,7-10], deficits in bone structure and development have been less studied. In this Short note, we describe the state of our knowledge on structural deficits in bone in RTT and in mouse models of RTT and provide a rationale for the treatment of bone fragility observed in this disorder.

Among the first reports of bone structural abnormalities was a 1995 radiological study that found reduced bone density in the hands of 86% of 17 girls with RTT [11]. In another study of 20 girls with RTT, bone mineral content and spine (bone) mineral density were significantly reduced in the RTT group; the authors concluded that girls with RTT were at risk for osteoporosis [12]. In 2001, Budden, et al. reported quantitative bone histomorphometry in three girls with typical RTT who required scoliosis surgery [13]. Bone volume and parameters of resorption (osteoclast surface and number) were decreased while surface parameters of formation (osteoid surface) were normal. The rate of bone formation was reduced in two of the girls. Shapiro, et al. found that 50% of girls with RTT had bone mineral density (BMD) levels that were more than two standard deviations below the norm [14]. These clinical studies prompted an evaluation of bone cell function.

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In 2009, using a *Mecp2*-null mouse model for RTT [15], O'Connor, et al. reported histological and biochemical studies on bone, which implicated both a defect in osteoblast function as well as chondrocyte abnormalities at the growth plate [16]. Femur length was shorter in *Mecp2*-null mice and overall skeletal size was reduced. The amount of trabecular bone was markedly decreased by age day 60 in *Mecp2*-null mice. Mineral apposition rates (MAR) were decreased in femoral trabecular bone and in calvarial bone. While loss of MeCP2 significantly reduced cortical, trabecular and calvarial bone volume compared with age-matched wild-type animals, MAR of cortical bone was not different from wild type. Furthermore, the number of osteoblasts/bone surface was significantly decreased in the *Mecp2*-null samples but osteoclast numbers appeared normal. Of interest, the deficiency in bone volume was evident before the onset of neurological symptoms in *Mecp2*-null mice.

Our recent micro-CT and histomorphometric studies of 5-week-old symptomatic *Mecp2*-null male and 8-week-old asymptomatic *Mecp2*-heterozygous (HET) female mice showed decreased numbers of osteoblasts but similar numbers of osteoclasts compared to WT, altered osteoblast morphology and decreased tissue synthesis of alkaline phosphatase in both *Mecp2*-null and HET mice compared to WT osteoblasts.

While it is known that MeCP2 can act to activate or repress gene transcription [6], the exact mechanisms by which MeCP2 regulates mRNA expression are largely unknown. Most of the studies related to MeCP2 effects on transcription have been done in brains/neurons rather than bones. Our previous baseline study of bone deficits in a mouse model of MeCP2 insufficiency to our knowledge is the first to report on the effects on osteoblast growth and on the expression of osteogenic transcription factors Osterix and Runx2 and the osteoblast marker type I collagen alpha 1 chain (Col1a1). Osteoblasts cultured from *Mecp2*-null mice, which unlike WT osteoblasts did not express MeCP2, had increased growth rates, but reductions in mRNA expression of Col1a1, Runx2 and Osterix compared to WT osteoblast [17]. The mechanism by which the loss of MeCP2 causes such defects is unknown. One mechanism by which MeCP2 may regulate transcription is via a unique epigenetic pattern of MeCP2 binding to methylated cytosine in a non-CG context recently described in neurons by Chen, et al. [18]. Whether such a mechanism is used in bone is an area for future research.

The fact that girls with RTT have osteoporosis as a consequence of defective osteoblast function raised questions regarding treatment to improve bone mass and lessen fracture risk. One available option was teriparatide (3-34 human recombinant PTH). However, current US FDA regulations do not allow children to be treated with teriparatide. The other option is zoledronic acid (ZA) a third generation nitrogen-containing bisphosphonate administered intravenously (thus avoiding difficulties in swallowing common to RTT) that currently is used in the treatment of osteoporotic disorders in children [19-21]. Clinical reports of the treatment of two patients with RTT with bisphosphonates have found positive effects on regression of osteoporosis and cessation of bone fractures [22,23].

In a recent study from our group, we administered ZA to MeCP2-deficient mice [24]. Treatment for male wildtype (WT) and mice lacking any MeCP2 (*Mecp2*-null) male mice began at 3 weeks of age, due to the limited life expectancy of the *Mecp2*-null mice. Mice heterozygous for *Mecp2* (HET) mice, which do not die early, began treatment at 8 weeks of age. Micro-computed tomography and dynamic analyses of bone turnover were performed after six weeks of treatment in both sets of mice. Zoledronic acid treatment led to highly significant increases in trabecular bone volume fraction, trabecular number, connectivity and apparent density of trabecular bone in all genotypes of mice [24]. Our findings on changes in trabecular bone on WT controls were similar to those of Zhu, et al. that examined the effects of different bisphosphonates on skeletal microarchitecture in immature C57Bl6/J male mice treated from 18 to 38 days of age [25]. Trabecular bone volume increased two-fold after ZA administration in both studies [24,25], indicating that zoledronic acid was able to improve trabecular bone even in the case of MeCP2 insufficiency. Our results suggested that the higher trabecular bone volumes were due to increased numbers of bone trabeculae and to smaller spaces between the trabeculae [24]. Trabecular thickness remained relatively unaffected [24,25]. Likewise, in women with osteoporosis treated yearly with ZA (Horizon Clinical Trial), iliac crest bone biopsy analysis by micro-CT showed improved trabecular bone function [26]. In our study, female mice also showed enhanced trabecular bone parameters after ZA treatment [24].

Cortical bone mass also is decreased in osteoporotic children with RTT [10,14], and clinical studies have shown increased cortical bone mass after ZA treatment in osteoporotic adults [27,28]. Our earlier baseline study also showed reductions in cortical bone thickness and in polar moment of inertia in male *Mecp2*-null mice [17]. However, in our ZA treatment study, ZA treatment had little effect on cortical bone [24]. We hypothesized that in the presence of MeCP2 deficiency, osteoblast responses to ZA inhibition of osteoclast activity in compartments such as periosteal and endosteal cortical bone differed from that in trabecular bone.

Recent bone studies of girls and women with RTT and age matched controls have shown significant reductions in the levels of biochemical bone markers, N-terminal pro-peptides of collagen type 1, C-terminal telopeptide cross links and osteocalcin in patients with RTT

[29,30], while both increases [29] and decreases [30] in bone-specific alkaline phosphatase have been reported in patients older than 25 years ($p < 0.001$) [29]. Our baseline micro-CT showed decreases in MAR, labeled bone surface (MS/BS) and in bone formation rate (BFR/BS) in HET and *Mecp2*-null compared to WT mice [17]. However, we also showed decreased MAR and MS/BS in HET females after ZA treatment raising the question of a potential negative effect on bone mass in treated females with RTT. However, the maintenance of bone mass with bisphosphonates in the limited number of patients reported supports the positive effect of ZA on bone mass in RTT [22,23].

Zoledronic acid is an antiresorptive agent that primarily decreases osteoclastic bone resorption, however, osteoblastic bone formation continues. As we note above, osteoblast protein synthesis is impaired in the presence of MeCP2 deficiency. Anticipated improvement in bone mass associated with a decrease in fracture rate is analogous to positive effect of zoledronic acid on osteoblast function in osteogenesis imperfecta. Recently, a consensus report on clinical guidelines for the management of bone health in RTT was published [31] that recommended the use of bisphosphonates for osteoporosis in these patients.

Conclusions

MeCP2 deficiency alters osteoblast directed bone formation in murine models and in young females with RTT [10,16,17,31-34]. Suppression of osteoclastic bone resorption with ZA leads to a net gain in trabecular bone at the proximal tibia, reflecting the decreased trabecular remodelling [35]. In contrast, treatment with ZA had little effect on cortical bone mass in the Bird mouse model of RTT [24]. These results support the limited clinical data discussed above suggesting that bisphosphonate treatment may improve bone strength in patients with RTT syndrome.

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