

Yoda1 augmented loading rescued bone properties after chemotherapy in adult mice

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INTRODUCTION: Anthracyclines such as doxorubicin are the backbone of chemotherapy regimens to treat various cancers, including the triple negative breast cancer.¹ However, chemotherapies adversely affected bone quality due to their off target effects on bone cells and the marrow.² For example, rats received anthracyclines showed reduction in the number densities of osteocytes and bone lining cells and significant increase in marrow adiposity.³ Our preliminary studies demonstrated that combining Yoda1, an agonist of mechanosensitive Piezo1 channel, with mechanical loading improved bone properties and protected bone structure in mice without and with breast cancer. Hence the aim of this study was to understand the efficacy of Yoda1 augmented exercise in mitigating bone impairment after chemotherapy in adult mice. The working hypothesis was that Yoda1 augmented loading could reduce bone loss caused by doxorubicin.

METHODS: To understand if Yoda1 augmented exercise can mitigate bone loss caused by doxorubicin (DOX), we intraperitoneally injected healthy aged mice (~50 weeks old) with doxorubicin at two doses: 2.5 mg/kg (n = 14) and 5 mg/kg BW (n = 8) for 6 times over Week 1 and Week 2. The control mice (n = 4) received DMSO vehicle injections at the same frequency. Yoda1 injection and tibial loading were applied 5 days per week over Week 3 to Week 5 to the low-DOX treated mice, and over Week 4 and Week 5 for the high-DOX treated mice, which received one week recovery in Week 3. Yoda1 was administered via intra-peritoneal injection 1 hr before the loading session. Yoda1 dose was varied with the DOX dose received by the mice, i.e., 2.5 mg/kg Yoda1 (n = 8) and DMSO vehicle (n = 6) for the low-DOX group and 5 mg/kg Yoda1 (n = 4) and vehicle (n = 4, among which 3 died in Week 3) for the high-DOX group. Tibial loading was applied at 4.5 peak load, 4Hz, 300 cycles per day, 5 days/week. The mice were sacrificed at the end of Week 5. This study was approved by IACUC. Cortical bone properties such as cortical polar moment of inertia (Ct.pMOI), bone resorption and bone formation were analyzed from the micro CT data at weeks 0, 2 and 5 at three locations: 0.35 mm and 3.15 mm above tibiofibular (T-F) junction (mid-diaphysis), and over a region of 2.1 mm under the growth plate (proximal end) where the trabecular bone property analysis was also performed. The changes between Week 5 and Week 2 were reported. Calcein bone label was injected 10 and 3 days before sacrifice. After sacrifice, the tibiae were chemically fixed and dissected at the T-F junction with the proximal ends being processed for paraffin embedding while the distal ends were used for cryo-embedding. Bone label analysis was done on the longitudinal cryo-sections, which were then stained to track enzymatic activities such as ALP and TRAP. Paraffin sections were used for H&E and TUNEL staining. femur bone shells were snap-frozen in liquid N₂ and used for RT-PCR analysis. To understand the acute effects of 2-week DOX treatment on gene expression, additional mice (3 no-DOX, 4 low-DOX, and 3 high-DOX treated) were prepared and femur shells used for RT-PCR as the mice described above. Mixed Model and one-way ANOVA were separately chosen for comparison within and between groups. For conservativeness, pairwise comparisons using Tukey HSD post hoc test was performed.

RESULTS SECTION: Doxorubicin administration resulted in a drastic decline in Ct.pMOI at the mid-diaphysis compared to control, which was reversed with Yoda1 treatment and tibial loading. Combined Yoda1 and tibial loading led to the greatest improvement of cortical bone structure with respect to the DOX non-treated group (Fig. A). Representative 2D slices of Week 5 and Week 2 cross-sections showed the prominent bone loss (red pixels) on endosteal surfaces in DOX treated mice and robust bone formation (green pixels) on periosteal surfaces in bones receiving Yoda1 and/or tibial loading (Fig. A). Interestingly, TRAP positive endosteal surface was significantly decreased five weeks after the initiation of DOX treatment, relative to no-DOX group, while this decrease was abolished with Yoda1 treatment in the high-DOX group (Fig. B). In the bone cortex, the % of apoptotic osteocytes was elevated from ~10% (control) to 47% (low-DOX) and 75% (high-DOX), while Yoda1 treatment showed a trend of suppressing the elevation in high DOX group (p = 0.17, Fig. B). The low-DOX treatment did not show appreciable acute changes in Wnt1, SOST, TRAP, RANKL/OPG expression level (Week 2) while Wnt, SOST and TRAP declined with time in DOX treated samples and Yoda1 showed no effect (Fig. C). In contrast, high-DOX treatment depressed SOST and greatly elevated TRAP and RANKL/OPG at Week 2 and the differences vanished at Week 5 (Fig. C).

DISCUSSION: Doxorubicin impaired cortical integrity and increased osteocyte viability as examined in two different doses. Sequential microCT tracking revealed that Yoda1 alone or in combination with loading showed beneficial effects countering the effects of doxorubicin. Despite of the diminished sensitivity of the mice in this study (50 weeks old) to mechanical loading, Yoda1 augmented loading successfully reversed the detrimental effect of doxorubicin. On cellular level, doxorubicin promoted catabolic bone turnover acutely while induced sustained osteocyte apoptosis. The latter, if not treated, would lead to a dysregulation of mechanosensing and homeostasis of bone⁴. The mechanisms by which Yoda1 and loading modulate bone's response to chemotherapy need further examination.

SIGNIFICANCE/CLINICAL RELEVANCE: Cancer is the leading cause of mortality and chemotherapy is an important component of standard care for cancer patients. However, chemotherapy induces off-target adverse effects on skeleton. This study explored the potential of a novel therapy to mitigate the detrimental skeletal effects caused by doxorubicin. We will study if the therapy preserves bone in cancer-bearing mice co-treated with chemotherapy.

References: [1] Schwartz, A. L. et al., 2007 [2] Zhou, J. Z. et al., 2016 [3] Robling, A. G. et al., 2020 [4] Lynda F Bonewald, 2011

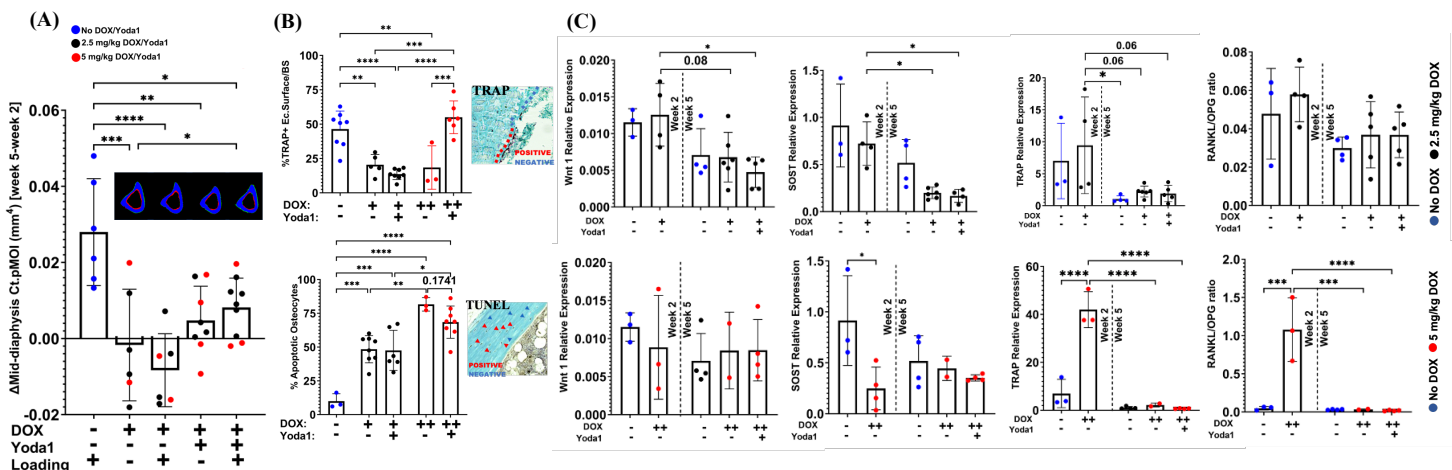


Figure 1. Effects of doxorubicin (DOX), Yoda1, and tibial loading on mature mice. (A) Yoda1+ loading countered the effects of DOX on cortical structural strength (pMOI). (B) TRAP and TUNEL staining of osteoclast activity and osteocyte apoptosis. DOX +: low dose; DOX ++: high dose (C) Gene expression 2 weeks or 5 weeks after initiation of doxorubicin treatment (housekeeping gene: ribosomal protein S2). (*p<0.01, **p<0.01, ***p<0.001, ****p<0.0001)