Introduction: Osteoporosis has been identified as one of four major late complications in the treatment of childhood cancers. The incidence of fractures in high-risk survivor groups ranges from 12-45%\(^1\). While the etiology is likely multifactorial, specific drugs have been implicated as potentially contributing by hormone-independent mechanisms. High-dose methotrexate (MTX) is one such drug that has been clinically implicated and has been reported to cause osteopenic changes in adult animals\(^2\). The effect of high dose MTX on bone density during growth has not been specifically studied, nor has the potentially therapeutic effect of bisphosphonates in this setting. The hypothesis to be tested was that high-dose MTX in a young growing rat model would result in reduction in bone mineral density (BMD) and that administration of the bisphosphonate alendronate (ALD) during and after the chemotherapy treatment would correct this adverse effect without further affecting growth.

Methods: Twenty-four weanling Sprague-Dawley (S-D) rats were randomly divided into 4 groups: CTL, MTX, ALD, or MTX+ALD. All procedures were approved by the institutional Committee for Humane Use of Animals. The control (CTL) and MTX (methotrexate only) groups received intra-peritoneal injections of either saline or methotrexate 0.75mg/kg/day, respectively, in a regimen of 5 days on/9 days off/5 days during the first 19 days of the study, according to a regimen previously used in a study of high-dose chemotherapy in an adult S-D rat model\(^2\). The ALD group received subcutaneous injections of the bisphosphonate alendronate (Merck), 0.30 mg/kg once per week for 6 weeks. The MTX+ALD group received both regimens. Six weeks after the start of the experiment, the animals were euthanized, and bone densitometry (BMD) was performed on the whole body and disarticulated hind limbs using DXA (small animal protocols, Lunar densitometry) and bone mass data which showed an increase beyond the precision of the data. The bisphosphonate findings are consistent with other densitometry and bone mass data which showed an increase beyond control following oral dosing of ovariectomized growing rats\(^1\) and following subcutaneous dosing in mechanically unloaded rats\(^4\). In the current experiment, the administration of alendronate more than reversed the loss of density caused by the MTX, suggesting that the dose used was potentially capable of counteracting larger osteopenic challenges. The observation of radiographic hyper-dense lines corresponding to weekly BP administration appears to be novel. Given the maintenance of normal growth and resultant limb lengths, these lines are unlikely to represent growth arrest lines, but rather episodes of reduced resorption of primary spongiosa and resulting apparent hypercalcification following each weekly bisphosphonate administration. It appears from this data that BP administration in the setting of pediatric chemotherapy may be valuable in preserving skeletal mass without further compromising growth.

Results: Densitometry showed a statistically significant mean 3.4% reduction in whole body BMD in the MTX group, compared to CTL (p<0.01) and a mean 17.9% decrease in femoral BMD (p<0.005), validating our model of chemotherapy-induced osteopenia in this skeletally immature animal model (Fig. 1). When the alendronate was administered during and after chemotherapy (MTX+ALD), there was a mean 6.9% increase in femoral BMD vs. control group (9.6% greater than MTX). Administration of alendronate alone resulted in 6.6% increase in BMD at 6 weeks. The MTX+ALD group received both regimens. Six weeks after the start of the experiment, the animals were euthanized, and bone densitometry and bone mass data which showed an increase beyond the precision of the data. The bisphosphonate findings are consistent with other densitometry and bone mass data which showed an increase beyond control following oral dosing of ovariectomized growing rats\(^1\) and following subcutaneous dosing in mechanically unloaded rats\(^4\). In the current experiment, the administration of alendronate more than reversed the loss of density caused by the MTX, suggesting that the dose used was potentially capable of counteracting larger osteopenic challenges. The observation of radiographic hyper-dense lines corresponding to weekly BP administration appears to be novel. Given the maintenance of normal growth and resultant limb lengths, these lines are unlikely to represent growth arrest lines, but rather episodes of reduced resorption of primary spongiosa and resulting apparent hypercalcification following each weekly bisphosphonate administration. It appears from this data that BP administration in the setting of pediatric chemotherapy may be valuable in preserving skeletal mass without further compromising growth.

Discussion: The reduction in BMD shown with this regimen of MTX establishes our model of chemotherapy-induced osteopenia in a skeletally immature animal model. The observed reduction in BMD with MTX was consistent with a previous morphometric study by Wheeler et al\(^1\) in 12 week old Sprague-Dawley rats in which bone formation was decreased and osteoclastic resorptive surface increased in response to the same regimen. The bisphosphonate findings are consistent with other densitometry and bone mass data which showed an increase beyond control following oral dosing of ovariectomized growing rats\(^1\) and following subcutaneous dosing in mechanically unloaded rats\(^4\). In the current experiment, the administration of alendronate more than reversed the loss of density caused by the MTX, suggesting that the dose used was potentially capable of counteracting larger osteopenic challenges. The observation of radiographic hyper-dense lines corresponding to weekly BP administration appears to be novel. Given the maintenance of normal growth and resultant limb lengths, these lines are unlikely to represent growth arrest lines, but rather episodes of reduced resorption of primary spongiosa and resulting apparent hypercalcification following each weekly bisphosphonate administration. It appears from this data that BP administration in the setting of pediatric chemotherapy may be valuable in preserving skeletal mass without further compromising growth.

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References:

Fig. 1 Group comparisons of bone density of the whole body and of the excised right femur. (+/− 1 S.D., *p<0.05)