Examination of the Effect of Black Cohosh on the Efficacy of Risedronate on Bone Mineral Density in an Ovariectomized Rat Model

Elysia A. Masters[1], A. Inselman[1], J. Moore[1], R. Agarwal[2], A. Gassman[3], G. Kuijpers[3], R. Beger[1], K. Delclos[4], S. Swift[5], L. Camacho[4], M. Vanlandingham[4], D. Sloper[1], N. Nakamura[1], G. Gamboa Da Costa[6], K. Woodling[4], M. Bryant[7], R. Trbojevich[7], Q. Wu[4], F. McLellen[7], D. Hansen[1], and D. Christner[2]

[1]Division of Systems Biology, National Center for Toxicological Research (NCTR), U.S. Food and Drug Administration (FDA), Jefferson, AR, [2]Office of New Drug Products, Center for Drug Evaluation and Research (CDER), U.S. FDA, Silver Spring, MD, [3]Division of Urology, Obstetrics & Gynecology, CDER, U.S. FDA, Silver Spring, MD, [4]Division of Biochemical Toxicology, NCTR, U.S. FDA, Jefferson, AR, [5]Scientific and Regulatory Affairs, CBD Industries, LLC, Charlotte, NC, [6]Office of the Center Director, NCTR, U.S. FDA, Jefferson, AR, [7]Office of Scientific Coordination, NCTR, U.S. FDA, Jefferson, AR.

Introduction

- Black cohosh is a top-selling, dietary supplement that has been marketed to relieve the vasomotor symptoms of menopause, and some studies suggest that it may protect against postmenopausal bone loss [1]. It is hypothesized that black cohosh may act by inhibiting RANKL signaling, thus preventing osteoclast activation and differentiation [2].
- Postmenopausal women are prescribed bisphosphonates, such as risedronate, to prevent osteoporotic bone loss. Bisphosphonates act by binding to hydroxyapatite where they accumulate in bone tissue and inhibit the bone resorption by osteoclasts, preventing bone loss and reducing fracture risk [3].
- It is not known whether there are pharmacodynamic interactions between these compounds when taken together, raising both safety and efficacy concerns.
- The aim of the study was to determine whether use of black cohosh, a dietary supplement, can affect the ability of the FDA-approved osteoporosis drug, risedronate, to protect against estrogen deficiency-mediated bone loss.

Methods

This study was approved by NCTR's IACUC. Female Sprague-Dawley rats (n=230) underwent bilateral ovariectomy (OVX) or sham surgery, then treated for 24 weeks with either vehicle, ethinyl estradiol, risedronate, black cohosh, or combination of risedronate and black cohosh, at low or high doses (Fig 1). Ethinyl estradiol, included as a positive reference control, was administered daily by oral gavage at 2.5 or 15 µg/kg of body weight. Risedronate was administered subcutaneously, twice weekly at 1.5 or 5 µg/kg. Black cohosh was administered daily by oral gavage at 10 or 100 mg/kg. Dose selection for black cohosh was based on literature that demonstrated a positive effect of the compound on bone with no reported toxicity [4, 5].

Treatment groups are detailed in Table 1. Bone mineral density (BMD) of the femur, tibia, and lumbar vertebrae was measured by dual-energy X-ray absorptiometry (DEXA) at weeks 0, 8, 16, and 24. At week 24, the liver, kidneys, and uterus were collected at sacrifice and weighed. Blood was collected for measurement of the bone markers, osteocalcin, alkaline phosphatase, and c-terminal telopeptide of collagen by ELISA.

Table 1. Treatment Groups and Dosing.

	Abbreviation	Treatment Group	Compound(s) Administered	Concentration	Route of Administration
1	Sham	Sham	CMC	0.5%	Oral Gavage
2	Veh	Vehicle (OVX)	CMC	0.5%	Oral Gavage
3	Lo EE2	Low Ethinyl Estradiol	Ethinyl Estradiol	2.5 µg/kg bw	Oral Gavage
4	Hi EE2	High Ethinyl Estradiol	Ethinyl Estradiol	15.0 µg/kg bw	Oral Gavage
5	Lo Ris	Low Risedronate	Risedronate	1.5 µg/kg bw	Subcutaneous
6	Hi Ris	High Risedronate	Risedronate	5.0 µg/kg bw	Subcutaneous
7	Lo BC	Low Black Cohosh	Black Cohosh	10 mg/kg bw	Oral Gavage
8	Hi BC	High Black Cohosh	Black Cohosh	100 mg/kg bw	Oral Gavage
9	Lo BC + Lo Ris	Low Black Cohosh + Low Risedronate	Black Cohosh Risedronate	10 mg/kg bw 1.5 µg/kg bw	Oral Gavage Subcutaneous
10	Lo BC + Hi Ris	Low Black Cohosh + High Risedronate	Black Cohosh Risedronate	10 mg/kg bw 5.0 µg/kg bw	Oral Gavage Subcutaneous
11	Hi BC + Lo Ris	High Black Cohosh + Low Risedronate	Black Cohosh Risedronate	100 mg/kg bw 1.5 µg/kg bw	Oral Gavage Subcutaneous
12	Hi BC + Hi Ris	High Black Cohosh + High Risedronate	Black Cohosh Risedronate	100 mg/kg bw 5.0 µg/kg bw	Oral Gavage Subcutaneous

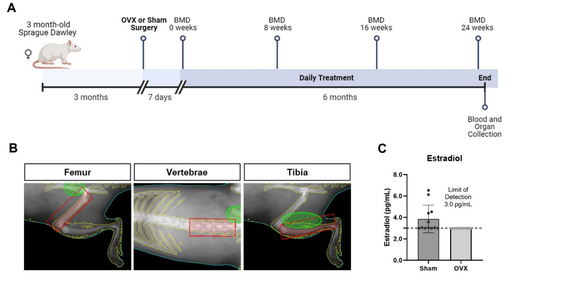

Figure 1: Study design to evaluate changes in bone quality in the ovariectomized rat. (A) Schematic of study design. (B) Representative DEXA images show regions of interest used to quantify bone mineral density (BMD) for the femur, lumbar vertebrae (L1-L4), and tibia in shaded red boxes. (C) Estradiol levels, measured by ELISA, were significantly greater in sham animals compared to OVX-vehicle animals (p = 0.0297, n = 12). The lower limit of detection in the assay was 3 pg/mL (dashed line).

Result

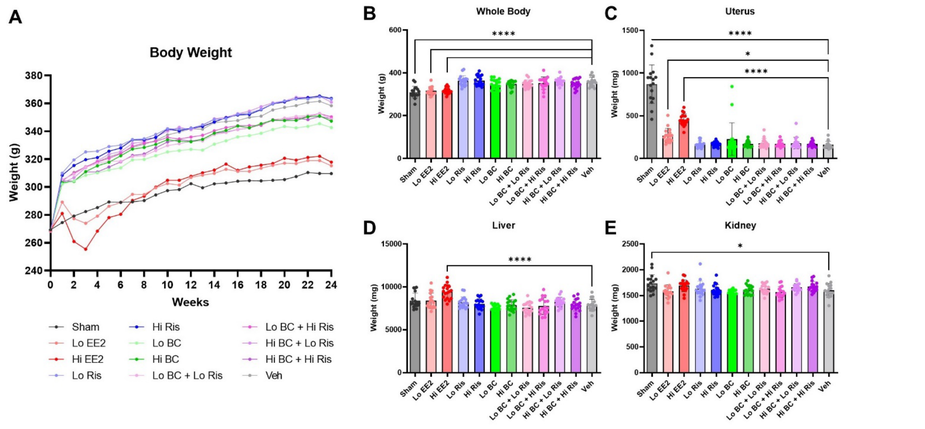

Figure 2: Longitudinal body weight and terminal body and absolute organ weight measurements. (A) Mean weekly body weight measurements. Due to significant reductions in body weight, doses of ethinyl estradiol were adjusted downward to 2.5 and 15 µg/kg body weight per day after three or four weeks of treatment for cohort one and two animals. Animals in cohorts three and four only received the lowered doses. (B) Sham surgery and EE2 treatment groups had significantly lower body weight measurements at week 24, while all risedronate and black cohosh treatment groups showed no significant differences compared to OVX-vehicle control. (C-E) Uterine, liver and kidney weights at week 24 showed no significant differences associated with treatments compared to OVX-vehicle.

Data plotted as mean ± SD. Significance was evaluated by one-way ANOVA with Sidak's post-hoc for multiple comparisons. * Indicates difference vs. OVX-veh; *p < 0.05, **p < 0.01, ***p < 0.001, ****p <0.0001; n = 16-18. EE2 = ethinyl estradiol; BC = black cohosh; Ris = risedronate sodium; Veh = OVX-vehicle control.

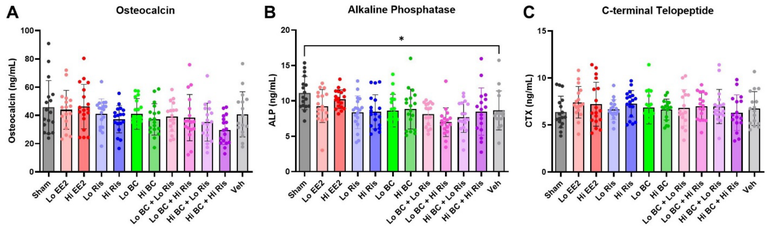

Figure 4: Black cohosh and risedronate coadministration had no significant effect on serum bone markers. Serum bone markers (A) osteocalcin, (B) alkaline phosphatase, and (C) C-terminal telopeptide were measured at sacrifice after 24 weeks of treatment. No statistically significant differences were observed in any treatment groups compared to OVX-vehicle control.

Data plotted as mean ± SD. Significance was evaluated by one-way ANOVA with Sidak's post-hoc for multiple comparisons; *p < 0.05; n = 16-18.

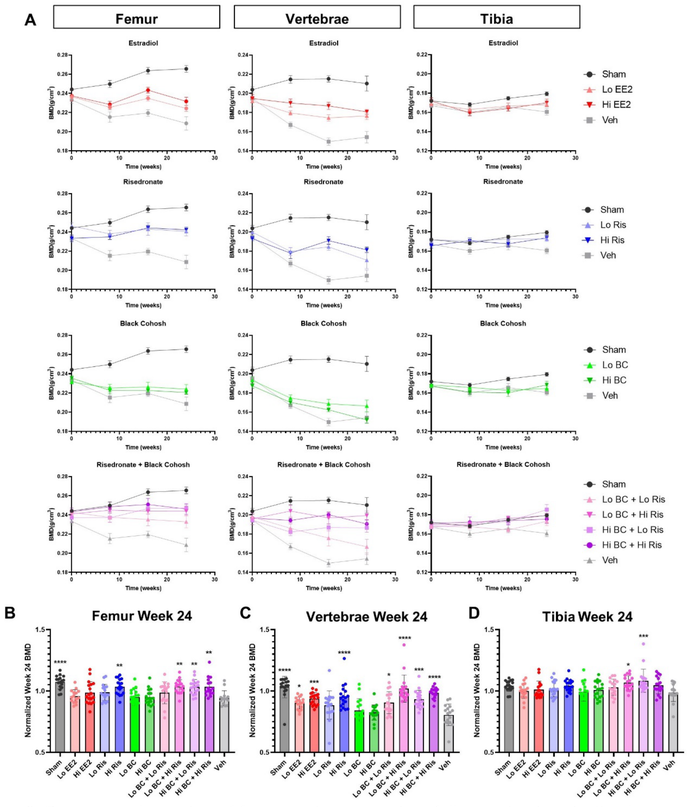

Figure 3: Bone mineral densities of femur, vertebrae and tibia following treatment with black cohosh and risedronate co-administration. (A) Measurement of BMD at 0, 8, 16, and 24 weeks of treatment. (B-D) BMD at week 24 was normalized to week 0. Normalized endpoint BMD was significantly greater in Hi EE2, Hi Ris and combined BC + Ris treatments compared to vehicle control. No statistically significant differences were observed between Lo Ris vs. Lo/Hi BC + Lo Ris or between Hi Ris vs. Lo/Hi BC + Hi Ris groups.

(A) Data plotted as mean ± SEM; n = 9-10. (B-D) Data plotted as mean ± SD. Significance was evaluated by one-way ANOVA with Sidak's post-hoc for multiple comparisons. *Indicates difference vs. Vehicle control; *p < 0.05, **p < 0.01, ***p < 0.001, ****p <0.0001; n = 14-18.

Conclusions

- Black cohosh alone or in combination with risedronate had no effect on body or organ weights, suggesting that this dose of black cohosh did not cause significant toxicity nor estrogenic activity.
- Dose-dependent prevention of BMD loss was observed in animals treated with prescription drug risedronate that is approved to treat osteoporosis caused by menopause.
- Treatment with black cohosh did not affect BMD or serum bone marker levels in OVX rats. Importantly, black cohosh administered in combination with risedronate did not negatively impact the positive BMD-enhancing properties of the oral-bisphosphonate risedronate.
- Due to the lack of standardization for black cohosh, the results and conclusions draw from this study are limited to the specific extract of black cohosh used. Since there was no efficacious benefit in BMD loss prevention with co-administration in this study, investigation of additional black cohosh extracts for additive or synergistic effects with bisphosphonates would be necessary to confirm claims of benefit.

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