Investigating the Impact of Ovariectomy- and Chemotherapy-induced Estrogen Loss in the Mouse Vagina

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The vagina is a complex tissue composed of an epithelium and a fibromuscular layer. When estrogen is lost during menopause, ovariectomy or gonadotoxic treatment such as chemotherapy, the vaginal anatomy and function is significantly altered resulting in sexual dysfunction, risk of infection and pain. The vaginal epithelium expands and shrinks during the course of the menstrual cycle (human) or estrous cycle (mice) and displays repair after childbirth. These functions indicate the vagina is a proliferative tissue and may contain stem cells that support vaginal regeneration. We hypothesize that chemotherapy has direct cytotoxic effects on the vagina, independent of ovarian toxicity, that cause estrogen-dependent degeneration of the vaginal epithelium. C57BL/6 mice (6 weeks old; n=60) were divided between four treatment groups (n=15/group): 1) ovariectomy (OVX) + chemotherapy (Chemo); 2) OVXVehicle; 3) ShamChemo; 4) ShamVehicle. Vaginal lavage was performed on all animals for 14 days to assess baseline estrus cyclicity prior to intervention. At 8 weeks, ovariectomy or sham surgery was performed. Ovary intact cohorts maintained normal estrous cycling, 2.53±0.74 and 2.60±0.74 cycles/14 days, and OVX animals lost cyclicity, 0.00 regular cycles/14 days, respectively ($p<0.05$). OVX animals also displayed significant weight gain that persisted throughout the trial ($p<0.05$). At 11 weeks of age, mice were injected with busulfan (24 mg/kg) and cyclophosphamide (200 mg/kg) or vehicle (DMSO + water). Reproductive tissues (n=5 mice/cohort) including vagina, cervix, ovary and uterus and serum were harvested at 7, 30, and 90 days post-injection (chemotherapy or vehicle). Chemotherapy did not have an immediate effect on estrous cyclicity in Sham animals (0.87±0.35 cycles/5 days). At 7 days post-injection, OVXVehicle and OVXChemo animals exhibited a significant decrease in reproductive tract weight compared with ShamVehicle and ShamChemo (2.88±0.38 and 2.57±0.32 vs. 7.65±1.56 and 7.43±1.63 tract weight(mg)/body weight(g), $p<0.05$). In addition, there were histologic differences in the OVX vaginas including alterations of the stroma, a loss of epithelial crypts, a significant reduction in epithelium area compared with ShamVehicle and ShamChemo ($p<0.05$). While there was no difference in the number of cells lining the basement membrane in the vaginal epithelium, we observed a significant reduction in Ki67+/DAPI+ nuclei on the basement membrane in OVXVehicle and OVXChemo animals (0.03+0.04 and 0.15+0.13 Ki67+/DAPI+ nuclei) compared with ShamVehicle and ShamChemo animals (0.54+0.25 and 0.57+0.05, $p<0.05$) suggesting cell proliferation is affected by OVX. Nerve growth factor receptor (NGFR) signal intensity in the basal epithelia was higher in the OVX animals (44.5±5.03, 37.0±9.75% area positive for NGFR signal) than the Sham animals (19.9±3.34, 27.2±14.7%). In addition, NGFR positive cells in the stroma were significantly reduced in OVX animals (0.52±0.79, 0.14±0.14% area positive for NGFR signal) compared to Sham animals (15.2±7.5, 18.2±6.2%, $p<0.05$). Taken together, we observed immediate effects of ovariectomy and associated estrogen loss to the reproductive tract, body weight and the epithelial and stromal compartments of the vagina. Data collection and analysis is ongoing for the 30 and 90 day timepoints; however, preliminary findings show a significant reduction in the ShamChemo reproductive tract weight

(5.05±0.68) compared to ShamVehicle (7.48±1.66) with no difference to the OVX cohorts (3.17±0.86 and 3.23±1.49 tract weight(mg)/body weight(g), respectively), suggesting chemotherapy impacts tissue integrity after 30 days ($p<0.05$). This work is supported by the Magee-Womens Research Institute and Foundation and the Richard King Mellon Foundation.