Abstract citation ID: deac107.450

P-478 Deleterious influence of cyclophosphamide on primordial follicles derives from increased DNA damage and reduced proliferation in xenotransplanted human ovarian tissue

L. Man, M.D.- M.Sc1, N. Lustgarten Guahmich1, E. Kallinos1, R. Bodine1, N. Zaninovic1, G. Schattman1, Z. Rosenwaks1, D. James1

1Weill Cornell medicine, Center for Reproductive Medicine, New York City, U.S.A.

Study question: What are the acute effects of cyclophosphamide (Cp) on primordial follicles (PrFs) in human ovarian tissue?

Summary answer: Administration of Cp damages PrFs via DNA fragmentation and results in reduced proliferation with no increase in PrF activation markers.

What is known already: Alkylating agents are highly gonadotoxic, and in cases where freezing oocytes, embryos, or ovarian tissue is impractical, a better understanding of the underlying damage mechanism could enable development of fertoprotective approaches (1). Studies from different groups suggest conflicting mechanisms of ovarian damage, with either PrF activation (2) or cell damage and apoptosis (3-4) proposed as drivers of PrF depletion. Few studies have examined this question using human tissue. We performed xenografts using ovarian tissue from a 17-month-old girl to ensure a graft with plentiful PrFs to test the acute effect of Cp.

Study design, size, duration: Cross-sectional study.

We utilized a xenotransplantation model in which human ovarian tissue is co-transplanted with endothelial cells (ECs) into immunocompromised mice (NSG) (5). Three weeks after xenotransplantation, time 0, intraperitoneal (IP) injection of (saline/Cp) was followed by an IP injection of ethynyl-deoxyuridine (EdU), at 0 and 24 hours, followed by 5-chloro-2'-deoxyuridine (CldU), at 48 and 72 hours. Grafts were harvested at 96 hours.

Participants/materials, setting, methods: We co-xenotransplanted human ovarian cortical tissue from a 17-month-old girl, cryopreserved for fertility preservation, into immunocompromised mice. After 3 weeks, Cp (75mg/Kg)/saline was administered IP. Mice were then sequentially injected with EdU, followed by CldU (both at 100mg/kg for 2 days) 24 hours apart. Twenty-four hours later, mice were sacrificed and xenografts were harvested and sectioned. Slides were stained for EdU, CldU, VASA, γ-H2AX, FOXO3a, and 4',6-Diamidino-2-Phenylindole-Dilactate (DAPI) and imaged using a confocal microscope.

Main results and the role of chance: We used anti-VASA staining to evaluate oocyte morphology, confirming that chemotherapy was not sterilizing; we counted 76.5% of morphologically normal PrF in the control group (Ctrl) (130/170) and 35.1% (13/37) in the Cp group. In the Ctrl group, 11% stained positively for DNA fragmentation using anti-γ-H2AX, whereas 43% were positive in the Cp group (p = 0.0073). To evaluate activation, we stained for FOXO3a in the oocyte nucleus. No difference was found between the groups: 52.4% (74/144) versus 44.6% (37/83) in Ctrl versus Cp, respectively. Interestingly, when comparing EdU incorporation, the Ctrl group had higher incorporation at 72%, versus the Cp group with 40% of incorporation...
(p = 0.0485), and no difference was found with CldU incorporation: Ctrl 22% versus 27% in the Cp group, respectively (P = 0.6960).

Limitations, reasons for caution: We measured acute effects, DNA fragmentation, and proliferation, at five days post-Cp administration; however, this falls short of evaluating processes that appear later (fibrosis and neovascularization). Also, the results may partly stem from the patient’s young age. Repeating the experiment using adult-derived tissue might yield different results.

Wider implications of the findings: A better understanding of both the timeline and underlying mechanisms that contribute to chemotherapy-related gonadotoxicity could diminish the damage and depletion of the ovarian reserve (PrF). These results provide evidence that direct damage to PrFs, and not increased activation, contributes to gonadotoxicity in the acute phase following administration of Cp.

Trial registration number: NA