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Abstract title:

LH prevents follicular damage and preserves the meiotic potential of oocytes exposed to chemotherapy at the primordial stage

Biography

Mr. Castillo completed his BSc in Biology with Health Sciences specialty at the Complutense University of Madrid (2016). He specialized in Reproductive Medicine through the MSc in Biotechnology of Human Assisted Reproduction and Embryology at the University of Valencia (UV, 2018). He is currently completing his PhD studies with PhD. Sonia Herraiz and Dr. Antonio Pellicer at IVI Foundation and Pediatrics, Obstetrics and Gynecology deparment at UV. He has been awarded with Spanish National Government grant (FPU16/05264). His research has focused in the female fertility preservation.

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Study question:

Does luteinizing hormone (LH) treatment protect follicle viability and meiotic potential of chemotherapy-exposed primordial follicles?

Summary answer:
LH improves the meiotic potential of murine metaphase II oocytes (MII-oocytes), avoiding the alkylating agents´ gonadotoxic effects, by the promotion of follicular DNA repair mechanisms.

What is known already:
High-dose chemotherapy with alkylating drugs induces detrimental changes on ovaries. Follicle viability is severely affected by DNA damage and apoptosis of oocytes and granulosa cells (GCs), leading to impaired follicular development and depletion. Early activation of DNA repair mechanisms, like homologous recombination through the ataxia-telangiectasia-mutated (ATM) pathway, is crucial for cell survival after cytotoxic events.
Previous results suggested LH treatment as an alternative for fertility preservation based on its protective role on the ovarian reserve against chemotherapy. Therefore, we aimed to assess the follicular protective mechanisms of LH and the meiotic potential of chemotherapy-exposed MII-oocytes in a mouse model.

Study design, size, duration:
Experimental study where twenty-four 7-week old CD-1 female mice were exposed to three experimental conditions (n=8/group): Control, chemotherapy (ChT) and ChT+LH. The ChT-treated groups were intraperitoneally injected with 12mg/Kg-busulfan and 120mg/kg-cyclophosphamide. The LH-treated animals received a pre-treatment dose with 1IU, 24 hours before ChT, followed by a second 1IU-dose administered with chemotherapy. Control-mice received saline. Ovaries from 6 animals/group were collected at 12 and 24 hours, while the remaining mice were maintained for 30 days.

Participants/materials, setting, methods:
The ATM-pathway, by Rad51 gene expression, was evaluated by RT-qPCR, while apoptotic (cleaved caspase-3) and anti-apoptotic (Bcl2) proteins were quantified by western-blot, on ovarian samples at 12

and 24h. Additionally, the 12h samples were screened for follicle DNA damage and apoptosis by γH2AX-staining and TUNEL-assay, respectively. The remaining animals were superovulated (10IU-PMSG + 10IU-hCG, 18 hours later) for MII-oocyte collection. Thus, spindle formation and chromosome disposition, referred to equatorial plate, were analyzed by confocal microscopy.

Main results and the role of chance:
LH treatment increased the expression of the DNA repair gene Rad51 at the 12h (Control: 1, ChT: 2.0±0.5; ChT+LH: 2.7±1.3; p=0.020 and p=0.019, respectively) and 24h timepoints (Control: 1, ChT: 0.5±0.5, ChT+LH: 1.4±0.3). The activation of the DNA repair signalling led to a rise of Bcl2/cleaved caspase-3 protein ratio, decreased by chemotherapy, enhancing cell survival on 24h-ovaries (Control: 2.5±0.9, ChT: 0.8±0.3, ChT+LH: 2.0±1.3).
Furthermore, LH treatment reduced the significant increase in the number of γH2AX-positive oocytes induced by chemotherapy (Control: 19.6±0.8%, ChT: 64.6±3.1%, ChT+LH: 42.6±4.1%; p=0.034 and p=0.021, respectively). Moreover, these positive effects were also observed in the GC integrity, by reducing the amount of follicles with >20% of TUNEL-positive GCs (Control: 1.6±0.8%, ChT: 8.5±0.8%, ChT+LH: 5.1±0.9%; p=0.034 and p=0.043), during the first 12 hours after treatment.
The meiotic potential, referring to MII-oocytes derived from follicles at the primordial stage during chemotherapy administration, was seriously affected in the ChT group, with a 34.5% decrease in spindle area (Control: 127.7±20.5μm^2, ChT: 83.7±7.7μm^2; p=0.019). Nevertheless, LH treatment was able to avoid this effect, preserving control-like values (ChT+LH: 119.7±2.7μm^2; p=0.034). Furthermore, LH diminished the number of MII-oocytes with at least one misaligned chromosome compared to ChT group (Control: 12.5%, ChT: 83.3%, ChT+LHx1: 58.3%).

Limitations, reasons for caution:
Although these findings represent the first steps of a new strategy for fertility preservation in cancer patients, this is an animal model study developed in mouse ovarian samples. Therefore, these results should be validated in order to properly identify the repairing mechanisms in a preclinical approach with human samples.

Wider implications of the findings:
LH treatment minimizes the deleterious effects induced by alkylating agents on follicular viability. The enhancement of DNA repair systems seems to be one of the main protective mechanisms promoted by LH. This improvement would contribute to produce MII-oocytes with increased potential to properly complete the meiosis II.

Keywords:
Fertility preservation
chemotherapy
Luteinizing hormone
Follicular protection
DNA repair mechanism