

Abstract Details

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LH in vitro treatment promotes DNA repair of mouse apoptotic follicles damaged by alkylating agents.

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Study question:

Does luteinizing hormone (LH) incubation protect mouse follicles against chemotherapy-induced apoptosis by promoting DNA repair during exposure to alkylating agents?

Summary answer:

LH promoted the homologous recombination mechanism allowing DNA repair to avoid follicle apoptosis in adult mouse ovaries cultured in vitro with alkylating drugs.

What is known already:

High-dose chemotherapy treatment with alkylating agents in female patients may impose deleterious effects on the ovary. The follicular pool can be destroyed by the induction of DNA damage leading follicles to atresia, if cells are not able to repair it. In oocytes, the homologous recombination via ataxia telangiectasia-mutated (ATM) pathway is a main mechanism for DNA damage response.

Previous studies suggested that LH incubation preserves the ovarian reserve against follicular depletion induced by chemotherapy in mouse models. We aim to elucidate if LH in vitro treatment protects follicles or promotes DNA repair mechanisms after ovarian exposure to alkylating agents.

Study design, size, duration:

Experimental in vitro study with 32 ovarian fragments obtained from eight 10 week-old CD1 mice. Ovarian fragments were randomly allocated to four experimental conditions (n=8/group): Control, Chemotherapy (ChT), ChT+LH-1IU and ChT+LH-5IU. Samples were conditioned with basal media for 24 hours before treatment. Tissue was pre-incubated with LH and one hour after, the ChT-treated groups were supplemented with alkylating drugs. Samples were recovered at 12 and 24 hours after treatments.

Participants/materials, setting, methods:

Ovarian fragments were in vitro cultured with α -MEM basal media (37°C-5%CO₂) After 24 hours medium was renewed, and treatments added. LH supplementation was performed 1h before treatment by adding 1 or 5IU of LH. Then, controls and ChT treated groups were supplemented with Vehicle or 1.2 μ M of busulfan +12 μ M of 4-Hidroperoxicyclophosphamide, respectively. Fragments were collected at 12 and 24h and analysed for follicle counts, TUNEL, apoptosis and DNA repair markers by western blot.

Main results and the role of chance:

LH incubation, especially at 5IU, reduced the number of TUNEL-positive-oocyte follicles (C: 74.2 \pm 17.2%; ChT: 90.4 \pm 6.7%; ChT+LH-1IU: 86.7 \pm 23.1%; ChT+LH-5IU: 63.1 \pm 16.5%, p=0.03) and the percentage of follicles with >20% of TUNEL-positive granulosa cells (C:68.1 \pm 40.8%; ChT:96.9 \pm 50.3%; ChT+LH-1IU:68.6 \pm 30.8%; ChT+LH-5IU:37.6 \pm 21.1%, p=0.04) already damaged after 24h of exposure to alkylating drugs. These decreases appeared during the first 12h of incubation although not statistically significant. Furthermore, both LH dosages reduced the percentage of apoptotic cells by follicle at all examined timepoints (12h exposure; C:56.3 \pm 6.3%; ChT:71.1 \pm 33.5%; ChT+LH-1IU:26.8 \pm 22.2%; ChT+LH-5IU:45.6 \pm 15.9% and 24h exposure C:

25.8±21.1%; ChT: 70.0±11.6%; ChT+LH-1IU: 61.9±29.2%; ChT+LH-5IU: 27.7±17.0%). The expression levels of the antiapoptotic protein Bcl2 were also quantified in all samples. LH incubation increased the levels of Bcl2 at 12h (C:0.2; ChT: 0.5; ChT+LH-1IU: 0.8; ChT+LH-5IU: 2.1) and 24h (C:1.1; ChT: 0.6; ChT+LH-1IU: 1.3; ChT+LH-5IU: 0.9).

In order to evaluate if the LH protective effect was mediated by DNA damage repair systems, ATM pathway proteins were examined. We found that both LH treatments increased levels of ATM (C:0.3; ChT: 1.1; ChT+LH-1IU: 1.9; ChT+LH-5IU: 2.1), BRCA1 (C:1.0; ChT: 1.3; ChT+LH-1IU: 2.2; ChT+LH-5IU: 1.5) and Rad51 (C:0.7; ChT: 1.3; ChT+LH-1IU: 2.0; ChT+LH-5IU: 1.3) from the first 12h of incubation.

Limitations, reasons for caution:

This is an in vitro study performed with mouse ovaries that should be confirmed in a preclinical study with a larger population of human samples prior to be proposed as a suitable clinical alternative to preserve fertility in cancer women.

Wider implications of the findings:

LH administration promotes follicle recovery by inducing DNA repair via the expression of proteins involved in the ATM pathway. To elucidate mechanisms and promote repairing might be crucial, as otherwise damaged follicles underwent atresia leading to follicular depletion and to ovarian failure.

Trial registration number:

Not applicable

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