The Ca^{+2} channel antagonist Verapamil exerts a protective effect against the negative actions of the mTOR inhibitor everolimus on ovarian follicle preservation

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ABSTRACT

The continuous activation of primordial follicles during the reproductive life of women leads to a gradual follicular loss, and finally, exhaustion of ovarian reserve at menopause. The PI3K/Akt/mTOR pathway is crucial for the proliferation and maturation of various types of cells, including ovarian follicular cells [1]. Pharmacological inhibitors of mTOR such as Everolimus, have been used in combination with anti-cancer therapies in order to preserve the pool of primordial follicles [2]. However, high doses or prolonged treatment with mTOR inhibitors may negatively affect follicular maturation. It has been shown that the combination with Verapamil, a P-glycoprotein and a CYP3A inhibitor, increases Everolimus effectiveness and allows the use of lower doses of the drug [3]. In previous studies we observed that their combined use increases the number of primordial follicles (*Pargianas et al., in preparation*). Here, we attempted to address, in a comparative manner, the molecular impact of the combination between Everolimus and Verapamil or Fisetin, an mTORC2 inhibitor, on the expression of genes that play important role in various stages of follicular maturation.

Ovaries isolated from female Wistar rats that received: Everolimus, Everolimus+Verapamil, Everolimus+Fisetin, and Fisetin, for 8 weeks were used. Total RNA was isolated and the expression levels of *SOHLH1/2, FIGLA, LHX8, ZP3, GDF9, AHR, YBX2, FSHR, CYP17A1, CYP19A1* and *STARD1* genes was monitored by RT-qPCR. We observed that the combination Everolimus+Verapamil increases the expression of genes responsible for primordial follicle maintenance and maturation as well as of genes that are important for the biosynthesis of steroid hormones and the response of ovarian cells to growth factors.

The data indicate that the combination of Everolimus with Verapamil helps the cells to respond properly to extracellular stimuli, thereby allowing a physiological and controlled maturation. These observations may encourage the use of such genes as potential biomarkers for the better investigation of the interventions that preserve ovarian follicle integrity.

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