INHIBITORY EFFECT OF LUTEOLIN ON FSH-INDUCED 17β -ESTRADIOL BIOSYNTHESIS

Azimova B.J.
Tashkent Pharmaceutical Institute, Tashkent city, Republic of Uzbekistan
e-mail: bax_gulim@rocketmail.com
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Relevance: In women of reproductive age, estrogen is a C18 steroid with diverse physiological roles, primarily produced by the granulosa cells of the ovaries through endocrine signaling. Its functions include promoting the development of secondary sexual traits, controlling gonadotropin release to trigger ovulation, preparing tissues for progesterone activity, preserving bone density, regulating lipoprotein metabolism, preventing urogenital atrophy, modulating insulin sensitivity, and supporting cognitive health. In healthy premenopausal women, 17β-estradiol (E2) is the predominant circulating estrogen, synthesized in the ovaries. This synthesis occurs through the aromatization of androstenedione into estrone (E1), which is subsequently converted into 17β-estradiol. During normal menstrual cycles, 17β-estradiol functions as a systemic hormone, exerting regulatory actions on distant target tissues.

Purpose of the study: to study of inhibitory effect of luteolin on FSH-induced 17β -estradiol biosynthesis in human ovarian granulosa cells and placental choriocarcinoma cells.

Materials and methods: Standard wells were prepared, blank wells and sample wells. Add 50-fold diluted standards to standard wells (dilution concentrations: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/mL), added 50 µL of standard and sample diluent to the blank wells and 50 µL of test sample to the remaining wells (all test samples and standards were prepared in duplicate during the test). µl of the working solution of antibodies were conjugated with the HRP enzyme, mix well, cover the plate with film and incubate at 37 °C for 60 minutes. All the liquid shakes off from the wells and blot them with clean absorbent paper. 350 µl of the washing solution was added to each well, soaked for 1 minute, shake off the liquid from the ELISA plate and blot dry. Repeat this washing step 5 times. After washing, immediately proceed to the next step without allowing the microplate to dry. µl of the substrate solution into each well, was covered the plate with film and incubate at 37 °C in the dark for about 15 minutes. The incubation time can be shortened or lengthened depending on the actual color development situation, but it should not exceed 30 minutes. When a clear gradient appears in the standard well (a clear blue gradient will appear in the first four color development wells), the assay could be stopped. Turn on the microplate reader 15 minutes before incubation to preheat. µl of stop solution was added into each well to stop the reaction. The order of adding the stop solution should be as close as possible to the order of adding the substrate solution. Immediately was measured the optical density (OD) value in each well at 450 nm using a multifunctional microplate reader.

Results: Previous our studies have shown that this compound can inhibit 17β-estradiol biosynthesis in human ovarian granulosa-like cells in a concentration- and time-dependent manner. However, it remains unclear whether luteolin regulates FSH-induced estrogen biosynthesis. The results show that luteolin exhibits a significant concentration-dependent effect, effectively inhibiting FSH-induced 17β-estradiol biosynthesis with an IC50 value of 2.36±0.27 µM.

Similarly, luteolin significantly inhibited FSK-stimulated 17β-estradiol biosynthesis at different concentrations KGN cells. The effect of luteolin on 17β-estradiol production in human placental choriocarcinoma JEG-3 cells was also investigated. Luteolin also significantly decreased 17β-estradiol levels in JEG-3 cells in a concentration-dependent manner. Compared with FSH-treated

cells, 10 μM luteolin inhibited 17β-estradiol production in JEG-3 cells by approximately 75%. Similarly, luteolin also significantly inhibited FSK-stimulated 17β-estradiol production in JEG-3 cells in a concentration-dependent manner.

Conclusions: These results suggest that luteolin was not cytotoxic to KGN and JEG-3 cells at the concentrations used to inhibit 17β-estradiol production in these cells, indicating that its inhibition of estrogen biosynthesis is not mediated by its cytotoxic effects. Luteolin inhibits 17β -estradiol biosynthesis in both human ovarian granulosa cells and placental choriocarcinoma cells.