Effects of gonadotrophin -releasing hormone agonists on apoptosis of granulosa cells

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Abstract

Granulosa cells are known to contribute to maturation of oocytes, and most of the growth factors exert their action via granulosa cells. It has been established that granulosa cell death during follicular atresia and luteolysis results from apoptosis. However, the precise mechanistic pathways of granulosa cell apoptosis have not yet been defined. In this study, we determined the proportions of apoptosis in granulosa cells treated with two kinds of gonadotrophin-releasing hormone agonists (GnRHa): buserelin and leuprorelin depot. The incidences of DNA fragmentation of human granulosa cells treated with buserelin and leuprorelin were 54.33% and 39.02%, respectively. The proportions of apoptotic bodies were 6.04% and 4.29%, respectively. There was a significant difference in the proportions of DNA fragmentation between the two kinds of Gn-RHa-treated granulosa cells. The apoptosis pathway and associated protein expression in granulosa cells treated with GnRHa were also determined. The Bax molecule, a pro-apoptosis protein, was expressed in granulosa cells undergoing apoptosis. In contrast, Bcl-2, an anti-apoptosis protein, could not be detected in the same group of granulosa cells. The distribution of cytochrome c determined via immunostaining showed a diffuse pattern, which most likely indicated that cytochrome c was translocated from mitochondria into the cytoplasm. Western blotting showed the expressions of caspase-9 and caspase-3 in patients' granulosa cells. The GnRHa effects on granulosa cells indicated a higher incidence of DNA fragmentation and apoptotic bodies in the buserelin-treated than in the leuprorelin depot-treated group. The granulosa cells go through the mitochondria-dependent apoptosis pathway; the Indicated pro-apoptosis protein Bax was expressed and induced cytochrome c release from mitochondria, which then activated caspase-9 and capase-3 until cell death occurred.