Leptin: pharmacological aspects in gynecology

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Summary

Hematic levels of leptin vary in relation to numerous metabolic factors and are able to interact in perfect synchrony with the hormones involved in the hypothalamus-pituitary-ovarian axis during the various phases of the reproductive cycle. In general it is maintained that the complex and multiple action mechanisms of leptin need to be clarified by further in-depth research studies.

It is likely that valid pharmacological applications of leptin will be found for human use although it is too premature to talk about concrete pharmacological answers and to formulate the relative complete technical protocols. In medicine the therapeutic use of leptin for humans has been reported in only a few cases. In fact human recombinant leptin has already been administered in gynecology for hypothalamic amenorrhea with precise protocols. In addition, very recent studies have provided the basis for new strategies to be developed concerning the use of leptin to fight multiple sclerosis.

At present there are considerable technical and economic problems in the production of leptin on a large scale. Most likely these problems will be overcome in the foreseeable future, and will involve new techniques related to genetics, cellular reprograming, and stem cells. In fact, new pharmacogenetic research has provided encouraging results for the production in industrial quantities of a more effective and fail-proof leptin.

Even considering that norms have not yet been proposed for pharmacological interventions with leptin for use directly on humans, in our work we have studied by immunohistochemistry methods the distribution of leptin and its receptor (Ob-R) in the ovaries of the female dog as a biological model, in the pre- and postpubertal phases and in other phases of the ovarian cycle. Given the hypothesis that the information obtained from immunohistochemical localization of the hormone and its receptor in various ovarian structures is transferable to humans, it could be useful to define therapeutic protocols based on the effective role of leptin and its receptor in folliculogenesis.

Key words: Leptin; Receptor; Ovarian cycle; Pharmacokinetics.

Introduction

Leptin is a glycolytic peptide consisting of 167 amino acids with a helicoidal structure that was discovered by Zhang *et al.* in 1944 [1]. Leptin is produced by white adipocytes and, as a very stable molecule, is put in circulation reaching target sites provided with specific receptors (Ob-R) belonging to the cytokine family. Ob-R is expressed in different tissues and with various receptor isoforms so that the biological activity of leptin is specifically regulated at the level of its receptor sites [2]. The Ob gene has been cloned and sequenced. In the mouse it is positioned on chromosome 6 whereas the homologous human gene is positioned on chromosome 7q31.3. The amino acid sequence of leptin in the mouse has 83% homology with the sequence in humans [3]. The mRNA of the leptin receptor is present in the anterior lobe of the hypophysis, in other areas of the brain and in various tissues such as the intestine, kidney, liver, spleen, lung, uterus, ovary, corpus luteum, thecal cells and ovarian follicular granulosa cells [4].

One of the reference models concerning pharmacokinetics and plasma leptin distribution in the tissues of various organs is the one described by Hill *et al.* [5]. By a two-dimensional equation they have also pointed out that the small intestine contains the highest leptin concentration (almost 4 times more) compared to the tissues of any other organ. Other researchers [6], through studies made on the pharmacological interaction of leptin, have found that this hormone is intensely connected with the proteins of the receptors of renal cell membranes of bovines. The precise role of leptin in female reproduction needs to be clarified.

Functional, pharmacological and genetic aspects of leptin

The main action mechanisms of leptin involved in female reproduction can be summarized as follows.

1. Leptin has very high, fluctuating concentrations during the day with an action mechanism controlled by the hypothalamic-pituitary-ovarian axis and not directly by the gonads [7].

2. Leptin levels in women are 40% higher than those in men, which is connected with the fat reserves necessary for the high-energy requirements of the various phases of pregnancy [8].

3. Leptin has a programed biological role and is sensitive to quantitative variations in every transitional phase of the ovarian cycle from childhood to puberty. In fact, leptin levels are low at birth and begin to rise during puberty. In particular leptin levels are higher in the luteal phase (11.4 ng/ml on average) than in the follicular phase of the cycle (10.0 ng/ml on average) ($p < 0.001$) [9].

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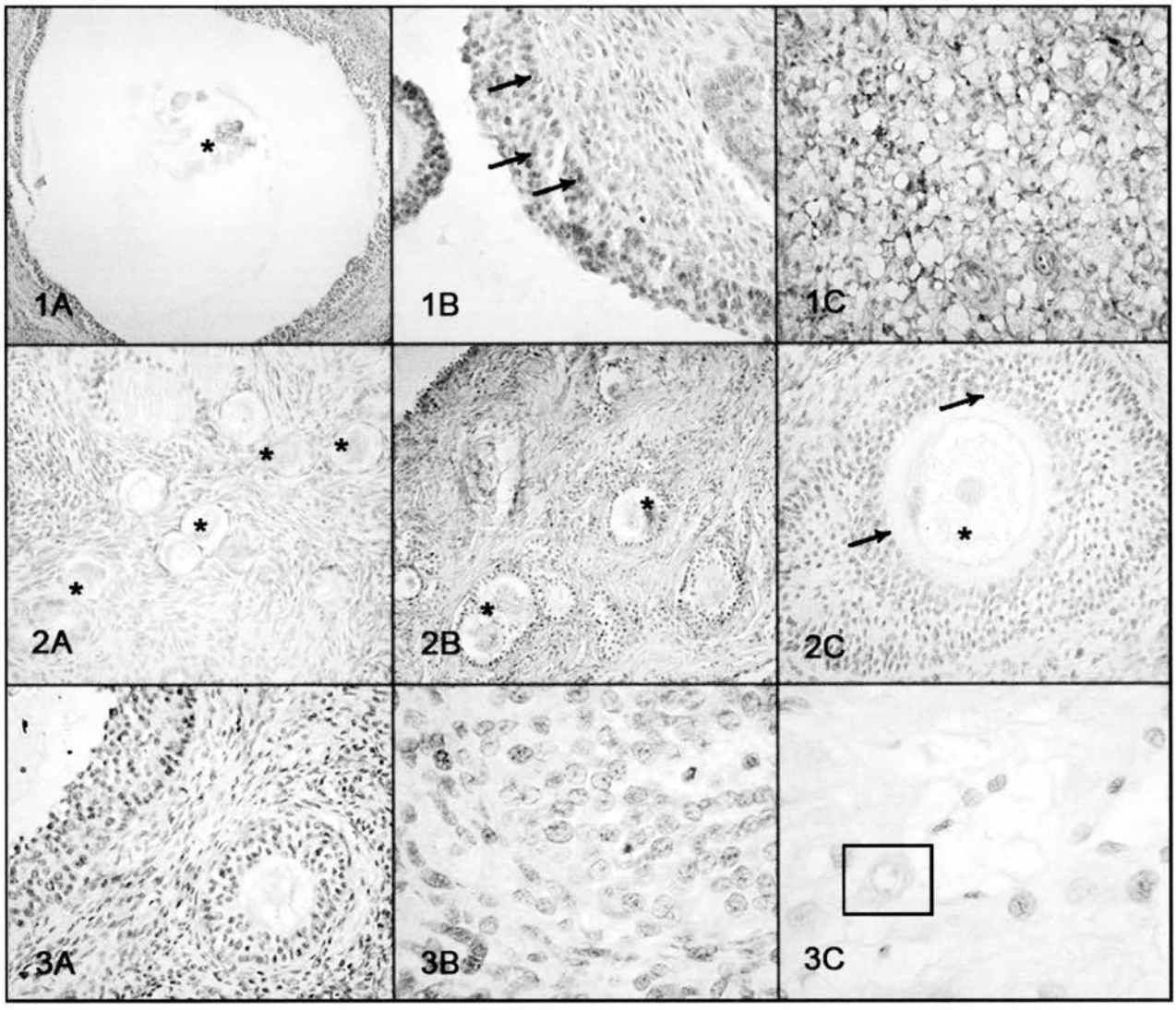

Figure 1A, 1B, 1C — Anti-OB immunoreactivity can be observed in the follicular fluid (1A, asterisk, 200x), in mature follicular granulosa cells (1B, arrows, 200x) and in the luteal cells of a corpus luteum (1C, 400x).
Figure 2A, 2B, 2C — Immunoreactivity can be observed in the preantral follicles (2A, 2B 200x, and 2C 400x), in the ovocytes (asteriks) and in the scattered granulosa cells (arrows).
Figure 3A, 3B, 3C — Ob-R immunoreactivity can be seen in mature follicular granulosa and thecal cells (3A, 400x and 3B, 1000x) and in luteinized granulosa cells (3C, 1000x).

4. Leptin directly controls the secretory activity of ovarian granulosa cells in women [10].

5. From in vitro studies it was found that leptin does not directly affect denuded oocyte maturation [11] while it does have a direct action on granulosa and thecal cells. Leptin modulates, through the intervention of correlated hormones, the processes of oocyte maturation, meiotic recovery, ovulation and formation of the corpus luteum [12].

6. Exogenous and systematic administration of leptin on the ovary can block ovulation [13].

7. Leptin regulates puberty at the hypothalamus level and not directly at the gonad level. The results show that leptin increases with age and reaches two peaks. The first peak signals the beginning of the first phase of puberty and the appearance of menarche, with a peak reaching 14.46 mg/8.85 ml. The second peak manifests at the end of puberty [14].

8. Women with low body weight and hypothalamic amenorrhea obtain improvement in reproductive function if they are treated with leptin. This is one of the few cases in the literature that points out the therapeutic administration of leptin as a base-line drug with a positive therapeutic response. The results obtained by administering recombinant human leptin to patients with hypothalamic amenorrhea for two weeks show that, within three months, levels of luteinizing hormone increase, frequency of LH pulsatility is regular, follicular diameter increases, dominant follicles increase in number, and ovarian volume and estradiol levels rise [15].

9. Obese women, due to quantitative imbalances of leptin, are subject to reproductive dysfunction with a high rate of polycystic ovary syndrome [16, 17].

10. Maternal hyperleptinemia is a characteristic of pregnancy in mammals, although all the roles of leptin and the mechanisms that control its synthesis seem to be species-specific. In both human and non human primate pregnancies leptin is produced by maternal adipose tissue, fetal tissue and placental trophoblasts. Specific receptors in uterine endometrium, trophoblasts and the fetus favor the direct effects of leptin on implantation, endocrine placental function and on the development of the product of conception. In addition to these effects on normal development, leptin is connected with mechanisms regulating different pregnancy-specific pathologies such as preeclampsia, gestational diabetes and intrauterine growth retardation [18].

11. Leptin concentrations increase proportionally with pregnancy progression; this has emerged from studies done on trophoblasts and placental villi during different phases of pregnancy in both humans and baboons [19].

12. Leptin levels increase during the first trimester of pregnancy and continue to change during the following periods. Thus they are correlated to the variations that normally take place during gestation, as increased weight with the deposit of adipose tissue. Leptin levels decrease quickly, immediately after delivery, and within 24 hours they return to the pre-pregnancy levels. Women in the first trimester, who are predisposed to spontaneous abortion, have considerably higher leptin levels than pregnant women who have a normal pregnancy [20].

13. Human leptin has been identified and purified in the epithelial cells of milk ducts in the period when colostrum is present and persists in the milk during the first few months of nursing. The newborn absorbs leptin from maternal milk that has not been degraded. Thus leptin plays an important role in the gastroenteric tract during breastfeeding even before it enters the circulation [21].

14. Premenopausal women who have normal cycles have higher leptin concentrations than postmenopausal women [22].

15. Hyperleptinemia is correlated with overweight and with insulin resistance syndrome in subjects that take antipsychotic drugs, especially clozapine [23]. Moreover during raloxiphene administration leptin levels increase [24]. Thus these drugs should be subject to strict drug monitoring because of the potential clinical problems that may develop in patients who consume them.

Material and Methods

Samples of ovarian tissue were collected from normal female dogs submitted to ovariectomy in the prepubertal and pubertal periods, the latter in different phases of the ovarian cycle. Ovarian fragments were fixed with 4% paraformaldehyde in PBS and prepared for light microscope study. Microtome sections were treated immunohistochemically using the rabbit polyclonal antibody for the product of the Ob gene that codes for human leptin (Santa Cruz, Biotech, CA). Leptin receptors were visualized using the Ob-R H300 rabbit polyclonal antibody (Santa Cruz, CA), recommended for studies of both long and short forms of leptin receptors in the mouse, rat, and man. The PAP method (peroxidase-antiperoxidase complex, Dako Cytomation) was employed to show the reactions, and appropriate negative and positive controls were carried out for both immunohistochemical reactions.

Results

The granulosa cells of mature luteinized follicles during the ovulation phase, and more markedly the corpus luteum, stained positive for leptin. The protein colored only faintly in the granulosa and thecal cell layers of immature follicles in every stage of the estrus cycle and in the prepubertal phase (Figure 1).

For leptin receptors, weak to strong reactivity was present in the oocytes of different sized preantral and antral follicles (Figure 2). Immunohistochemical localization of Ob-R was scattered mainly at the level of the granulosa and thecal cells in growing follicles in proestrus ovaries (Figure 3). Ob-R staining in granulosa and thecal cells is most probably ascribable to membrane receptor internalization. No Ob-R immunoreaction was detected in the corpus luteum.

In the biological model of the domestic animal, leptin undoubtedly sends a metabolic signal to the reproductive system through direct action on the granulosa and thecal cells in follicles at different stages of development.

In conclusion, the presence and regulation of the production of leptin and its Ob-R receptors during the estrus cycle of the female dog modulates steroidogenesis and contributes to oocyte maturation.

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