# Effects of Analogs of Luteinizing Hormone-Releasing Hormone (LHRH) on the Hypophysis-Gonad Axis and Tumor Growth

Theses of PhD Dissertation

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## INTRODUCTION

The discovery of LHRH has had a major impact on medicine and has led to a variety of clinical uses of LHRH and its analogs in gynecology and oncology. The development of different LHRH analogs (agonistic, antagonistic, cytotoxic) has also influenced the medical research of a broad spectrum, including the approach to better understanding the physiology of reproduction.

Our work was also inspired by a double goal. **First (I.),** we investigated the effects of antagonistic analogs of LHRH on the hypophysis-gonad axis, **second (II.),** we examined the toxic and antitumor effects of a targeted cytotoxic analog of LHRH (AN-207).

**I.** Since many data about the diverging pattern of luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretion in intact and gonadectomized animals have emerged in the past two decades (FSH-releasing factor?, FSH-RF), we focused our attention on studying FSH secretion after treatment with antagonistic analogs of LHRH. Our aim was to provide new data on the hypothalamic regulation of FSH secretion. With the use of potent LHRH antagonists, we could eliminate the receptor-mediated effect of endogenous LHRH on gonadotropin secretion and were able to investigate the portion of FSH secretion that is independent from LHRH. We studied:

**I./1.** Inhibitory activity of LHRH antagonists (MI-1544 and SB-030) on gonadotropin secretion in dispersed **rat pituitary cell superfusion system** *in vitro*

**I./2.** the effect of **single and chronic administration** of antagonistic analog of LHRH (MI-1544) on gonadotropin secretion in ovariectomized **(OVX) rats**

**I./3.** the effect of **chronic administration** of antagonistic analogs of LHRH (MI-1544 and SB-030) on the hypophysis-gonad axis in **intact rats**

**I./4.** the presence of a presumed FSH-RF in the median eminence of

**OVX rats** *in vivo*, by combining **LHRH antagonist treatment** (MI-1544, s.c.) with **electrochemical stimulation (ECS)** of the median eminence of the hypothalamus

**I./5.** the presence of a presumed FSH-RF in the median eminence of **OVX rats** *in vitro*, in the **hypothalamus-hypophysis coperifusion system**

**II.** A novel class of LHRH analogs, the family of targeted cytotoxic analogs of LHRH, is being developed in an endeavor to reduce the toxic side effects and increase the efficacy of antineoplastic agents by delivering them more selectively to tumor cells possessing receptors for LHRH. We investigated the *in vivo* effect of a targeted cytotoxic analog of LHRH (AN-207) on the growth of a human prostate cancer expressing LHRH receptors, along with the general toxic effects of this analog, in nude mice. Because gonadotroph cells of the pituitary express high affinity receptors for LHRH, it is also important to determine whether cytotoxic LHRH analogs would damage pituitary function. The conjugate (AN-207) we used consists of a derivative of doxorubicin (2-pyrrolinodoxorubicin, AN-201) linked covalently to carrier molecule [D-Lys$^6$]LHRH. We examined:

**II./1.** the **toxic effect** of targeted cytotoxic LHRH analog AN-207 on **pituitary function in rats** *in vitro* and *in vivo*

**II./2.** the **antitumor effect and toxicity** of therapy with AN-207 in **nude mice** bearing s.c. xenografts of PC-82 human androgen dependent prostate cancer possessing receptors for LHRH



## RESULTS

### I./1. Inhibitory activity of LHRH antagonists MI-1544 and SB-030 on gonadotropin secretion in dispersed rat pituitary cell superfusion system *in vitro*

Both antagonists (MI-1544 and SB-030) prevented the LH and FSH releasing effect of exogenous LHRH in the superfusion system. This inhibition was three times more prolonged on LHRH-induced FSH release than on LHRH-induced LH release. When administration of LHRH antagonists preceded the administration of LHRH (preincubation), 10-20-times lower concentrations of antagonists were efficient in producing the same inhibitory effect that was obtained when given along with LHRH at the same time.

### I./2. Effect of single and chronic administration of LHRH antagonists on secretion of pituitary gonadotropins in *OVX rats*

**a.** Single injection of MI-1544 (10 or 100 μg/animal) resulted in an instant and long-lasting decrease of the elevated serum LH concentration of OVX rats, whereas concentration of serum FSH did not change within 6 hours after the injection. MI-1544 given at the higher dose decreased serum FSH only 12 hours after administration.

**b.** Chronic treatment for 21 days with MI-1544 (10 μg/day) in OVX rats markedly decreased serum LH concentration while serum FSH did not change. During treatment, pituitary LH concentration increased significantly but pituitary FSH concentration remained unchanged.

### I./3. Effect of chronic administration of LHRH antagonists on the pituitary-gonad axis in *intact rats*

**a.** Chronic treatment for 21 days with MI-1544 or SB-030 (10 μg/day) in intact rats suppressed serum LH concentration to an undetectable level, while serum FSH concentration remained unchanged. In the same animals, pituitary LH concentration significantly increased while FSH concentration decreased by the end of the treatment.

**b.** Serum progesterone levels decreased markedly during the experiment but serum estradiol concentrations remained unchanged. The regular cycling of rats was interrupted, and the weight of ovaries decreased significantly in groups treated with either antagonist. The mean uterus weight slightly increased (MI-1544) or remained unchanged (SB-030), and the weight of pituitaries was unaffected by the treatment.

### I./4. Effect of ECS of the median eminence preceded by LHRH antagonist treatment on serum LH and FSH concentrations in *OVX rats*

ECS of the median eminence evoked significant elevations of both serum LH and FSH concentrations in OVX rats, as measured at 10 and 60 min after ECS. Administration of LHRH antagonist MI-1544 (100 μg/rat) 60 min prior to ECS prevented the ECS-induced raise of serum LH but only slightly diminished the ECS-induced elevation of serum FSH concentration.

### I./5. *In vitro* release of LH and FSH from dispersed pituitary cells, induced by substances released from mediobasal hypothalami fragments

*In vitro*, in the hypothalamus-hypophysis coperifusion system, both exogenous LHRH and factors released from stimulated fragments of mediobasal hypothalami (MBH) evoked LH and FSH release from dispersed pituitary cells. After administration of LHRH antagonist MI-1544, exogenous LHRH could not induce FSH release and evoked only a small release of LH. However, the medium that had perfused the stimulated MBH fragments, induced FSH release without eliciting LH release.



### II./1. Effect of targeted cytotoxic analog of LHRH on pituitary function *in rats*

The loss in body weight of rats treated with injection of cytotoxic analog of LHRH AN-207 at a dose of 150 nmol/kg i.v. did not differ significantly from that of rats treated with cytotoxic radical AN-201 at a dose of 75 nmol/kg.

**a.** In the superfusion system *in vitro*, one week after treatment, we detected a selective reduction in LH secretion of pituitaries derived from rats injected with AN-207. This reduction was accompanied by a slight decrease in pituitary GH and TSH secretion. At the same time, cytotoxic radical AN-201 caused a non-selective damage to LH, GH and TSH secreting cells. Three weeks after treatment, normal secretory responses were found in both groups.

**b.** *In vivo*, 2, 4 and 6 weeks after treatment, basal serum LH, GH and TSH levels did not differ from controls in groups injected with either AN-207 or AN-201. In the AN-207 treated group, the specific LH, GH and TSH responses to LHRH, GHRH and TRH stimuli were also similar to controls at all time points. In the AN-201 treated group, 2 weeks after treatment, the LHRH-induced LH response was moderately decreased. The GHRH-induced GH and TRH-induced TSH responses were unchanged at 2, 4 and 6 weeks after treatment, as compared with controls.

### II./2. Antitumor activity and toxicity of cytotoxic LHRH analog AN-207 in *nude mice* bearing xenografts of human prostate cancer PC-82 expressing receptors for LHRH

**a.** Single i.v. injection of AN-207 at a dose of 200 nmol/kg did not cause a significant weight loss of nude mice as compared with controls, while single i.v. injection of cytotoxic radical AN-201, given at the same dose, resulted in a significant decrease in body weight. Evaluation of total leukocyte and platelet counts also showed that cytotoxic radical AN-201 had a significantly greater toxic effect on nude mice than conjugated cytotoxic LHRH analog AN-207.

**b.** Treatment with conjugate AN-207 resulted in a remarkable

suppression of growth of PC-82 tumors throughout the 8 weeks of experimental period, whereas cytotoxic radical AN-201 produced only a moderate decrease in volume of PC-82 tumors during the first 4 weeks. In control mice, carrier molecule [D-Lys$^6$]LHRH alone or in an unconjugated mixture with AN-201 was ineffective in tumor growth inhibition.

## CONCLUSIONS AND NEW OBSERVATIONS

1. Under physiological conditions, regulation of pituitary FSH secretion is controlled mainly by factor(s) other than LHRH.

2. Factor(s), other than LHRH, involved in the regulation of FSH secretion, can be found in, and its (their) release can be evoked from the median eminence of the hypothalamus.

3. Targeted cytotoxic LHRH analog AN-207 is highly selective for cells possessing LHRH receptors and less toxic to others, while its cytotoxic component, doxorubicin derivative AN-201, causes a non-selective damage to cells of the recipient.

4. The damage in pituitary function caused by cytotoxic LHRH analog AN-207 and cytotoxic radical AN-201 is reversible followed by a full recovery.

5. Administration of cytotoxic LHRH analog AN-207 can result in a long-lasting and marked reduction in growth of tumors expressing receptors for LHRH, raising the possibility of treating LHRH receptor-positive cancers efficiently with targeted cytotoxic analogs of LHRH.

## MERIT OF RESULTS

Our experiments using antagonistic analogs of LHRH provided new data on the regulation of pituitary gonadotropin secretion.

The hypothalamus-hypophysis coperifusion system, designed by us, made it possible to investigate the effect of endogenous releasing



factors on pituitary gonadotropin secretion, under dynamic *in vitro* circumstances.

Data obtained from experiments with cytotoxic LHRH analog AN-207 may contribute to expanding our knowledge of *in vivo* and *in vitro* effects of targeted cytotoxic LHRH analogs, a novel class of anticancer agents.

## LIST OF PUBLICATIONS

Journal articles:

1. **Koppán M.**, Nagy A., Schally A.V., Plonowski A., Halmos G., Arencibia J., Groot K.: Targeted cytotoxic analog of luteinizing hormone-releasing hormone AN-207 inhibits the growth of PC-82 human prostate cancer in nude mice. ***The Prostate*** 1998; (accepted)

2. **Koppán M.**, Kovács M., Mező I., Flerkó B.: Electrochemical stimulation of the median eminence evokes FSH but not LH release after LHRH antagonist treatment *in vivo* and *in vitro*. ***J Neuroendocrinol*** 1998; 10:231-236.

3. Kovács M., Schally AV, Nagy A., **Koppán M.,** Groot K.: Recovery of pituitary function after treatment with a targeted cytotoxic analog of luteinizing hormone-releasing hormone (LH-RH). ***Proc Natl Acad Sci USA*** 1997; 94:1420-1425.

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Abstracts:

**7.** **Koppán M.**, Schally A.V., Nagy A., Halmos G., Arencibia J.: Citotoxikus LHRH analóg gátolja az LHRH receptor-pozitív PC-82 prosztata karcinóma növekedését *in vivo*. *A Magyar Endokrinológiai és Anyagcsere Társaság XVII. Kongresszusa*, Pécs, 1998.

**8.** **Koppán M.,** Kovács M., Mező I., Teplán I., Flerkó B.: Effects of a potent LHRH antagonistic analog on the FSH release in rats. *4th World Congress of Gynecological Endocrinology*, Madonna di Campiglio, Italy, 1995. ***J Gynecological Endocrinol*** 1995; **9**(Suppl1): P6.

**9.** **Koppán M.**, Kovács M.: LH-RH antagonista analóg hatása az eminentia mediana elektrokémiai stimulációját követő LH és FSH release-re. E-8, p 17. *A Magyar Endokrinológiai és Anyagcsere Társaság XV. Kongresszusa*, Budapest, 1994.

**10.** Kovács M., **Koppán M.**, Mező I., Teplán I., Flerkó B.: Effects of new highly potent GnRH antagonists on the pituitary-gonad system. *Satellite Symposium on Gonadotropins, GnRH, GnRH Analogs and Gonadal Peptides*, Paris, 1992.