Urol Res (1984) 12:175–178

Urological Research

Unresponsiveness of the Reproductive Organs of the Male Mouse to Treatment with a Potent Luteinizing Hormone-Releasing Hormone Agonist (ICI-118,630)

G. J. van Steenbrugge, J. C. Romijn, F. H. de Jong[1] and F. H. Schröder

Departments of Urology and [1]Biochemistry (Division of Chemical Endocrinology), Erasmus University Rotterdam, Rotterdam, The Netherlands

Accepted: October 24, 1983

Summary. The effects of administration of a luteinizing hormone-releasing horomone (LHRH) analogue (ICI-118,630) on plasma concentrations of hormones and weights of reproductive organs were studied in male mice. A single injection of the analogue (54 μg/kg body weight) caused a transient elevation of the concentration of LH in the plasma, up to 7 times the level in control mice. Daily administration of the analogue for a period of 3 weeks caused a small but significant decrease of the weight of the ventral prostate. The weight of the seminal vesicles in mice treated with the analogue, however, did not differ from that in control animals. The concentration of testosterone and LH in the plasma of these mice was not significantly different from those in control mice. It is demonstrated that the LHRH-analogue has no "castration-like" effect on the accessory sex organs of the male mouse, which is therefore not a suitable model in the study of these compounds.

Key words: LHRH-agonist, Mouse, Prostate, Seminal vesicles.

Introduction

Long-acting analogues of LHRH have been shown to cause marked and prolonged release of gonadotropins but also have paradoxical inhibitory effects on reproductive function via an inhibition of the secretion of gonadal steroids in female and male animals [3, 8].

Although the antifertility effects are now well documented in man [5], rat [3], dog [16] and monkey [1], data obtained with mice are rather scarce [2, 7].

The LHRH-analogue, ICI-118,630, was shown to be effective in reducing plasma estradiol levels and decreasing the weight of ovary and uterus in female rats [10]. Furthermore this compound caused a regression of the growth of chemically induced mammary carcinomas [11] and of prolactin-secreting pituitary tumors in the rat [9]. Inhibition of the growth of prostate tumors in two experimental rat models [12] as well as in patients with prostatic carcinoma [15] was achieved after treatment with LHRH-agonists.

The human prostatic tumor line PC-82, is serially transplantable on nude mice, and its growth is dependent on androgens [6, 13]. Therefore it would be important to study the effectiveness of LHRH-agonists in suppressing the growth of PC-82 tumor tissue.

As a pilot study for this investigation we studied the effectiveness of the potent LHRH-agonist (ICI-118,630) in suppressing plasma testosterone levels and causing a reduction in the weight of accessory sex glands in heterozygous male nude mice.

Materials and Methods

Peptide

The LHRH-analogue used in this study was D-ser-(Bu^t)^6-AzGly^{10}-LHRH (ICI-118,630, ICI-Holland, Rotterdam, The Netherlands) [4]. The peptide was dissolved in citrate buffer and administered as a daily intraperitoneal injection (100 μl) between 1300–1400 h. Control animals received citrate buffer only.

Animals

Heterozygous male nude mice, bred in our institute, were used for this study. The animals, separately housed, were kept in a 12 h light/12 h dark cycle and had access to food and water ad lib. Humidity was controlled automatically (60%).

For these experiments mice with an age of 20–24 weeks and a mean body weight of 35 g were used. Castration was carried out via the scrotal route under light ether anaestesia.

Animals treated for a 21 day period were sacrificed 24 h after the last injection. Trunk blood was collected after decapitation. After centrifugation at 2,000 *g* for 15 min, plasma samples were stored at −20 °C until analyzed. A number of pituitaries of control mice and mice treated with the analogue was frozen for the estimation of LH and prolactin.

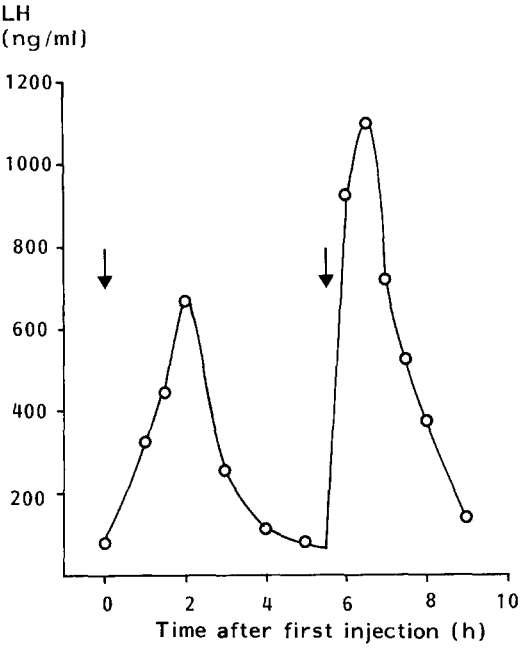

Fig. 1. Plasma LH-levels in mice receiving intraperitoneal injections with 1.9 μg ICI-118,630. Each time point represents the value of one single mouse. The times of the first and second injection are indicated by *arrows*

For these experiments the pituitaries were thawed and homogenized in 0.5 ml saline with a potter Elvehjem homogenizer.

Plasma Hormone Assay

Prolactin (PRL) in pituitary extracts was measured by a heterologous radioimmunoassay system, using materials and protocols supplied by the NIAMMD Rat Pituitary Hormone Distribution Program. LH in plasma and pituitary extracts was measured by radiommunoassay [18]. The results were expressed in terms of NIAMDD-rat LH-RP-1.

Plasma levels of testosterone were estimated using the radioimmunological procedure described by Verjans et al. [17].

Statistical Procedure

The significance of difference between values of different groups was calculated using two-tailed Student's T-tests. Differences were considered to be statistically significant when P was smaller than 0.05.

Results

Short-Term Effect of the Analogue on Plasma LH

Short-term effects on plasma LH levels after a single intraperitoneal injection of 1,9 μg (54 μg/kg body weight) ICI-118,630, were examined in a time course experiment. Since repeated blood sampling from mice was impracticable, every point of this curve was derived from plasma of individual mice. A rapid increase of immunologically reactive LH in the plasma was found (Fig. 1). A peak in the plasma concentration (700 ng/ml) was reached at 2 h after administration of the compound. After this transient elevation, control levels of LH were reached within 5 h after the first injection. A second injection with a similar dose again showed a rapid increase up to peak plasma levels above those reached after the injection (Fig. 1).

Table 1. Plasma hormone levels in male mice treated for 21 days with a daily injection of the LHRH-agonist, ICI-118,630 (54 μg/kg body weight) compared to control and to castrated animals. Values are expressed as means ± S.D., with the total number between parentheses

Group	Testosterone (ng/ml)	LH (ng/ml)
Control	8.0 ± 8.7 (12)	46 ± 8 (7)
ICI-118,630	2.8 ± 3.0 (12)	46 ± 6 (10)
Castration	<0.01[a] (11)	263 ± 155[a] (9)

[a] Significantly different from control group ($P < 0.01$)

Effects After a 21-Day Treatment Period

The effect of daily doses of 54 μg/kg bw on reproductive organ weight and plasma hormones in intact male mice was studied after 21 days of treatment, and compared with data obtained after castration during the same period. Table 1 shows the plasma levels of testosterone and LH in the different groups. A decrease of the plasma concentration of testosterone in the mice treated with the analogue was observed; the difference to the controls, however, was not statistically significant. It can also be seen from Table 1 that the decrease in circulating testosterone was not as extensive as that observed in the castrated animals.

Plasma LH concentrations were similar in both the control mice and those treated with the analogue. Castration during the same period resulted in significantly elevated LH levels (Table 1). Table 2 shows the effects of castration and treatment with the analogue on the wet weight of several organs as compared to those of control mice. The compound caused a slight but significant ($P < 0.01$) decrease of the weight of the ventral prostate compared to the controls. The weight of the seminal vesicles and those of testes and pituitaries, however, were identical in both groups. It is also shown in Table 2 that the decrease of the weight of the ventral prostate in the mice treated with the analogue was not as extensive as that observed in the castrated group. In the latter group the mean wet weight of the ventral prostate, the seminal vesicles and the pituitary showed a significant decrease ($P < 0.01$) compared to those in the control group. The body weight in the group of mice treated with the analogue was not significantly decreased. This decrease was also not significantly different from that in the control mice, while the weight change after castration was significantly different ($P < 0.05$) from that in control mice (Table 2).

Table 2. Body and organ weights of male mice treated for 21 days with a daily injection of ICI-118,630 (54 μg/kg body weight) compared to control and castrated animals. Values are expressed as means ± S.D., with the total number between parentheses

Organ	Controls	ICI-118,630	Castration
Ventral prostate (mg)	18.6 ± 2.9 (11)	14.6 ± 2.5[a] (11)	4.1 ± 0.7[a] (11)
Seminal vesicles (mg)	379 ± 70 (10)	379 ± 84 (10)	45 ± 10[a] (12)
Testis (mg)	200 ± 14 (10)	209 ± 27 (10)	–
Pituitary (mg)	1.8 ± 0.2 (11)	1.8 ± 0.2 (11)	1.2 ± 0.3[a] (12)
Initial body weight (g)	38.4 ± 1.1 (11)	35.2 ± 3.5 (12)	37.1 ± 5.1 (12)
Final body weight (g)	36.7 ± 1.9 (11)	34.1 ± 3.6 (12)	33.1 ± 4.4 (12)
Weight Change (%)	4.7 ± 5.1 (11)	2.9 ± 4.3 (12)	10.7 ± 3.8[a] (12)

[a] Significantly different from control group ($P < 0.01$)

Table 3. Concentrations of prolactin and LH estimated in pituitary extracts of mice treated for 21 days with LHRH-analogue, ICI-118,630, compared to control animals. Values are expressed as means ± S.D.

Group	*n*	Prolactin (ng/ml)	LH (ng/ml)
Controls	5	106 ± 13	1,326 ± 340
ICI-118,630	5	119 ± 29	823 ± 449

Table 3 shows the concentration of PRL and LH in pituitary extracts after treatment with ICI-118,630. The concentration of PRL in the mice treated with the analogue was identical to that in the control group, while the LH concentration was slightly, but not significantly declined in the analogue treated group.

Discussion

The present study was conducted to investigate the effect of a LHRH-agonist on the release of LH from the pituitary, and the envisaged "castration-like" effect on the reproductive organs of the male mouse.

Figure 1 shows that a single injection of the analogue (ICI-118,630) caused a rapid and marked elevation of plasma LH. This result proved that the mouse pituitary is responsive to the LHRH-agonist, which is in agreement with earlier observations with the same compound in rats [10, 11].

Daily administration of the analogue for a period of 3 weeks resulted in mean levels of plasma LH that were similar in the analogue-treated and control groups, while castration resulted in a marked and prolonged elevation of LH during the same period (Table 1). In the analogue-treated group the mean testosterone level was slightly but not significantly decreased compared to that in the vehicle-treated, control animals. It was not unexpected, however, that the levels of LH were similar in both groups since a single dose of the analogue resulted in only a temporary elevation of LH (Fig. 1) and since the blood of the mice in this experiment was obtained 24 h after the last injection. As a consequence of the protocol used for this study the content of LH and PRL in the pituitary was also determined 24 h after the last dose. Nevertheless lower values of LH in the pituitary extracts were found after treatment with the agonist; the difference, however, was not statistically significant.

The tendency towards a lower content of LH in the pituitary might indicate that long-term treatment with the agonist does not result in a desensitization of this organ.

In a study with rats Maynard and Nicholson [10] observed that after a treatment period of 14 days this agonist elicits a marked and extended release of LH in the plasma.

After the treatment period of 3 weeks we observed a modest, but significant decrease of the weight of the ventral prostate compared to that in control mice (Table 2). The weights of the seminal vesicles, however, were similar in the analogue-treated and control mice. This result could be explained by insufficient suppression of testosterone (Table 1). Furthermore it can not be ruled out that the agonist exerts a direct effect on the ventral prostate.

This resistance of the male mouse gonads to the inhibitory effects of LHRH-analogues has been demonstrated previously by Bex et al. [2]. In their study administration of another potent analogue (Wy-18,481) to adult male mice had no effect on the weight of the testis or ventral prostate, and stimulated that of the seminal vesicles.

In the same study it was shown that with the same treatment protocol a much lower dose of the compound had a significant effect on the reproductive organs in the rat.

It has also been shown that mouse Leydig cells do not respond to various LHRH-analogues in vitro, while the presence of binding sites for LHRH-like peptides could not be demonstrated [7]. In contrast rat Leydig cells were shown to possess receptors for LHRH-like peptides [14], and to respond markedly to treatment with LHRH-agonists [3, 8].

The latter results and those of the present study indicate that treatment with a LHRH-agonist has no suppressive effect on the testis of the mouse. Although it can not be excluded that a different dosing schedule or another route of

administration could lead to different results, our findings support those with other agonists that the susceptibility of the mouse to treatment with these compounds is quite different from that in other species.

It is concluded that the mouse is not an appropriate model to study the "castration-like" effects of LHRH-agonists.

Consequently these compounds are not applicable for the treatment of hormone-dependent prostatic tumors transplanted on nude mice. In spite of the latter conclusion, it would be worthwhile to investigate whether LHRH-agonists exert a direct effect on human prostatic tissue.

Acknowledgements. We wish to thank ICI (Rotterdam, The Netherlands) for the generous gift of ICI-118,630. Dr. S. W. J. Lamberts (Department of Medicine) is thanked for the estimation of prolactin in the pituitary extracts, Mr. Piet Kreeft for his technical assistance and Dr. M. A. Blankenstein for the critical evaluation of the manuscript.

References

1. Akhtar FB, Wickings EJ, Zaida P, Nieschlag E (1982) Pituitary and testicular functions in sexually mature rhesus monkeys under high-dose LHR-agonist treatment. Acta Endocrinol 101: 113–118
2. Bex FJ, Corbin A, France E (1982) Resistance of the mouse to the antifertility effects of LHRH agonists. Life Sci 30:1263–1269
3. Bowers CY, Humphries J, Wasiak T, Folkers K, Reynolds GA, Reichert LE (1980) On the inhibitory effects of luteinizing hormone-releasing hormone analogues. Endocrinology 106: 674–683
4. Dutta AS, Furr BJA, Giles MB, Valcaccia B (1978) Synthesis and biological activity of highly active α-aza-analogues of luliberin. J Med Chem 21:1018–1023
5. Faure N, Labrie F, Lemay A, Bélanger A, Goudreau Y, Laroche B, Robert G (1982) Inhibition of serum androgen levels by chronic intranasal and subcutaneous administration of a potent luteinizing hormone-releasing hormone (LH-RH) agonist in adult men. Fertil Steril 37:416–424
6. Hoehn W, Schroeder FH, Riemann JF, Joebsis AC, Hermanek P (1980) Human prostatic adenocarcinoma: some characteristics of a serially transplantable line in nude mice. Prostate 1:95–104
7. Hunter MG, Sullivan MHF, Dix CJ, Aldred LF, Cooke BA (1982) Stimulation and inhibition by LHRH analogues of cultured rat Leydig cell function and lack of effect on mouse Leydig cells. Mol Cell Endocrinol 27:31–44
8. Labrie F, Bélanger A, Cusan L, Seguin C, Pelletier G, Kelly PA, Reeves JJ, Lefebvre FA, Lemay A, Gourdeau Y, Raynaud JP (1980) Antifertility effects of LHRH agonists in the male. J Androl 1:209–228
9. Lamberts SWJ, Uitterlinden P, Zuiderwijk-Roest JM, Bons-Van Everlingen EG, de Jong FH (1981) Effects of a luteinizing hormone-releasing hormone analogue and tamoxifen on the growth of an estrogen-induced prolactin-secreting rat pituitary tumor and its influence on pituitary gonadotropins. Endocrinology 108:1878–1884
10. Maynard PV, Nicholson RI (1979) Effects of high dose of a series of new luteinizing hormone-releasing analogue in intact female rats. Br J Cancer 39:274–279
11. Nicholson RI, Maynard PV (1979) Anti-tumor activity of ICI 118,630, a new potent luteinizing hormone-releasing hormone agonist. Br J Cancer 39:268–273
12. Redding TW, Schally AV (1981) Inhibition of prostate tumor growth in two rat models by chronic administration of D-Trp^6 analogue of luteinizing hormone-releasing hormone. Proc Natl Acad Sci USA 78:6509–6512
13. Romijn JC, Oishi K, van Steenbrugge GJ, Bolt-de Vries J, Schröder FH (1982) Some studies on the characterization of a transplantable androgen-dependent human prostatic carcinoma (PC-82). Proc Third Int Workshop on Nude mice. Gustav Fischer, New York
14. Sharpe RM, Fraser HM (1980) Leydig cells receptors for luteinizing hormone releasing hormone by administration or deprivation of the releasing hormone. Biochem Biophys Res Commun 95: 256–262
15. Tolis G, Ackman D, Stellos A, Metha A, Labrie F, Fazekas ATA, Comaru-Schally AV (1982) Tumor growth inhibition in patients with prostatic carcinoma treated with luteinizing hormone-releasing hormone agonists. Proc Natl Acad Sci USA 79:1658–1662
16. Vickey BH, McRae GI, Bonasch H (1982) Effect of chronic administration of a highly potent LHRH agonist on prostate size and function on geriatric dogs. Prostate 3:123–130
17. Verjans HL, Cooke BA, de Jong FH, de Jong CMM (1973) Evaluation of a radioimmunoassay for testosterone estimation. J Steroid Biochem 4:665–676
18. Welschen R, Osman P, Dullaert J, de Greef WJ, Uilenbroek JThJ, de Jong FH (1975) Levels of follicle-stimulating hormone, luteinizing hormone, oestradiol-17β and progesterone, and follicular growth in the pseudo-pregnant rat. J Endocrinol 64:37–47

Dr. G. J. van Steenbrugge
Erasmus University
Department of Urology
Laboratory for Experimental Surgery
P.O. Box 1738
NL-3000 DR Rotterdam