Use of analogues of luteinizing hormone-releasing hormone for the treatment of cancer

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Introduction

When research on luteinizing hormone-releasing hormone (LH-RH) and analogues started in many pharmaceutical companies, it was predicted that these agents would be used for the treatment of hypogonadal states in human clinical medicine and for the regulation and improvement of fertility in veterinary medicine. The major objective, however, was to produce an antagonist of LH-RH which could be used primarily as an antifertility agent in men and women. Like a number of other biologically potent molecules the unexpected results provided by LH-RH and its agonistic analogues changed both the impetus and direction of research.

The finding that potent analogues of LH-RH would prevent puberty when given continuously to immature female rats and would cause ovarian and uterine atrophy in mature rats (Johnson, Gendrich & White, 1976; Vale, Rivier, Rivier & Brown, 1977) prompted a rapid re-appraisal of the therapeutic potential of these agents. The paradoxical anti-gonadal effect was not confined to the female since potent analogues of LH-RH were shown to prevent sexual maturation in the male, to reduce the weights of the testes and accessory sex organs, and to arrest spermatogenesis and decrease plasma androgens (Oshima *et al.*, 1975; Auclair, Kelly, Labrie, Coy & Schally, 1977; Vale *et al.*, 1977; Pelletier *et al.*, 1978; Tcholakian *et al.*, 1978; Rivier, Rivier & Vale, 1979).

Although prevention of conception remains a very important potential use for potent LH-RH analogues, a number of problems will have to be overcome before such agents reach the stage of extensive clinical trial. In the shorter term exploitation of the castration-like effects produced by LH-RH analogues will be in the treatment of precocious puberty, endometriosis and benign and malignant diseases of the breast and prostate. Since clinical trials are now beginning with these agents in the treatment of hormone-responsive tumours, it is timely to describe data from tumour models in animals which support the belief that analogues of LH-RH may well prove clinically effective.

Rat mammary tumours induced by dimethylbenzanthracene (DMBA)

Huggins, Grand & Brillantes (1961) showed that administration of DMBA to young female rats resulted in the formation of mammary tumours. This so-called DMBA-induced mammary tumour model has proved most valuable for the study of hormone-dependent cancer because it responds to a range of ablative and additive endocrine therapies that are effective in women with breast cancer. Ovariectomy (Huggins *et al.*, 1961; Manni, Trujillo & Pearson, 1977; Fiebig & Schmahl, 1977), hypophysectomy (Daniel & Pritchard, 1963), pharmacological doses of oestrogens (Pearson, Molina, Butler, Llerena & Nasr, 1972), antioestrogens (Desombre &

Arbogast, 1974; Nicholson & Golder, 1975; Jordan, 1976; Jordan & Koerner, 1976; Manni *et al.*, 1977), progesterone (Huggins *et al.*, 1961) and cytotoxic agents (Fiebig & Schmahl, 1977) all produce tumour regression. The observations of Danguy *et al.* (1977) and Desombre, Johnson & White (1978) that D-Leu⁶, des Gly¹⁰, Pro-ethylamide⁹-LH-RH also caused marked regression of DMBA-induced mammary tumours prompted a number of studies with ICI 118630, D-Ser (Bu⁶), Azgly¹⁰-LH-RH, an analogue approximately 50–100 times as potent as the natural deca-peptide at inducing ovulation in constant-oestrous, androgen-sterilized rats (Dutta, Furr, Giles & Valcaccia, 1978a).

In all studies the tumours were measured by calipers along the longest axis and the axis perpendicular to it. In an initial experiment (Dutta, Furr, Giles, Valcaccia & Walpole, 1978b), 6 rats were given s.c. injections for 28 days, starting 104 days after administration of DMBA, of saline or ICI 118630 (5 µg/rat twice daily on weekdays and 5 µg/rat daily on Saturdays and Sundays). Tumour numbers and area, calculated from the product of the two measurements, were recorded weekly. The results shown in Text-fig. 1 demonstrate a clear regression of tumours in animals treated with ICI 118630 compared with a marked increase in size in the controls. A complete disappearance of the tumour occurred in 5 cases and no new tumours were found in the treated group. In contrast 8 new tumours were palpated in the control group by the end of the 28-day dosing period (Table 1).

Text-fig. 1. Effect of subcutaneous treatment twice daily for 28 days with 5 µg ICI 118630 on the total tumour area in 6 rats with DMBA-induced mammary tumours. Six rats given vehicle served as controls.

Table 1. Change in numbers of DMBA-induced mammary tumours in rats following subcutaneous treatment twice daily with 5 µg ICI 118630

Time after dosing (days)	Number of measurable tumours	
	Control	118630 treated
0	11	13
7	12	13
14	13	10
21	16	8
28	19	8

A more detailed study was then performed (Nicholson & Maynard, 1979). Five weeks after administration of DMBA, rats were palpated for tumours at weekly intervals and the tumour volume was calculated using the formula $(4/3)\pi r^3$, where r is the mean radius. When tumours reached an appropriate size (1.76 cm^3 ; 1.5 cm mean diameter) a small portion ($\sim 100 \text{ mg}$) was removed under ether anaesthesia and stored at -20°C for oestrogen receptor analysis (Nicholson, Davies & Griffiths, 1978). The animals were divided into 6 groups and treated for 20 days as follows.

Group I. Eleven animals bearing 18 tumours received twice daily i.m. injections of $5 \mu\text{g}$ ICI 118630 in 0.1 ml saline (9 g NaCl/l).

Group II. Seven animals bearing 9 tumours received twice daily injections of $0.5 \mu\text{g}$ ICI 118630 in 0.1 ml saline.

Group III. Eight animals bearing 12 tumours received twice daily injections of $0.05 \mu\text{g}$ ICI 118630 in 0.1 ml saline.

Group IV. Seven animals bearing 13 tumours received twice daily injections of 0.1 ml saline.

Group V. Nine animals bearing 12 tumours were ovariectomized at the start of the dosing period and were then given twice daily injections of 0.1 ml saline.

Group VI. Nine animals bearing 12 tumours received daily i.m. injections of $100 \mu\text{g}$ of the antioestrogen, tamoxifen, in 0.1 ml sesame oil.

After completion of the treatment period regrowth of the tumours was monitored at weekly intervals and animals in Groups I–III and VI were ovariectomized when at least one tumour in each animal reached a tumour volume of more than 8.2 cm^3 (2.5 cm mean diameter).

Blood samples were obtained from the tumour bearing animals at the end of the treatment period and plasma oestradiol was estimated by radioimmunoassay (Maynard & Nicholson, 1979).

Text-fig. 2. Response of oestrogen receptor-positive DMBA-induced rat mammary tumours to treatment with ICI 118630 and tamoxifen. Groups I to III were injected twice daily for 20 days with the doses of ICI 118630 shown; Groups IV and V received 0.9% (w/v) saline and Group V was ovariectomized at the start of the treatment period. Group VI was given $100 \mu\text{g}$ tamoxifen daily.

Text-fig. 3. Growth patterns of DMBA-induced rat mammary tumours during and after cessation of treatment with ICI 118630. (a) Ten animals with oestrogen receptor-positive tumours given 5 µg ICI 118630 twice daily for 20 days. The points are the mean values and the range is shown by the stippled area. (b) Tumour regrowth after the cessation of treatment. (c) Further responses to ovariectomy.

All tumours assessed in the study were classified according to their oestrogen receptor (ER) content. Oestrogen receptor-positive tumours were defined as those containing more than 8 fmol ER/mg cytosol protein. Using this classification 78% of tumours were oestrogen receptor positive. Data on the effect of treatment on these tumours are shown in Text-figs 2 and 3. Doses of 5 and 0.5 µg ICI 118630 were as effective as ovariectomy and 100 µg tamoxifen in causing tumour regression over the 20-day treatment period. Reducing the dose of ICI 118630 to 0.05 µg markedly decreased its effectiveness.

After the end of treatment no further growth was seen in 5 tumours in animals treated with 5 µg ICI 118630. Of the remaining 10 tumours, 2 showed a most marked increase in volume during the 20-day period following treatment (Text-fig. 3). Tumours which regrew after treatment with ICI 118630 retained hormone dependency and regressed again after ovariectomy (Text-fig. 3).

The growth patterns of 7 oestrogen receptor-negative tumours in animals treated with ICI 118630 or tamoxifen are shown in Text-fig. 4. Five tumours continued to grow but 2 tumours in animals treated with ICI 118630 regressed. In the three instances when ovariectomy was performed after the cessation of treatment, a progressive tumour growth pattern was maintained (Text-fig. 4). During the study, 2 fibroadenomas were detected; both were unresponsive to ICI 118630 treatment and ovariectomy.

In addition to the effects observed on tumour growth, ICI 118630 also decreased the number of new tumours appearing during treatment (Table 2). The effect was clear even at the lowest dose level of 0.05 µg ICI 118630. When treatment ceased, some growth of new tumours did occur, although the total number of tumours found was less than in the control group. The growth patterns of the newly formed tumours after cessation of treatment are shown in Text-fig. 5. Of the 11 tumours examined, 9 were detected within 21 days of drug withdrawal. Where ovariectomy was performed, it was shown that all 7 tumours were hormone-dependent (Text-fig. 5). Similar results were observed in the group treated with tamoxifen.

Twice daily treatment with 5 µg ICI 118630 for 20 days significantly ($P < 0.001$) reduced plasma oestradiol concentrations to values indistinguishable from those of ovariectomized

Text-fig. 4. Response of oestrogen receptor-negative, DMBA-induced mammary tumours to treatment with ICI 118630 and tamoxifen. (a) Animals injected twice daily for 20 days with 5 µg ICI 118630 (●), or 0.5 µg ICI 118630 (○) or daily with 100 µg tamoxifen (■). (b) Effect of ovariectomy on growth response in 3 animals.

Table 2. Effect of ICI 118630, tamoxifen and ovariectomy on the development of DMBA-induced mammary tumours in rats

Group	Dose/injection (µg)	No. of animals	New tumours formed		Total
			During treatment	After treatment	
I ICI 118630	5.0	11	0	8	8
II ICI 118630	0.5	7	0	3	3
III ICI 118630	0.05	8	4	4	8
IV Control	—	7	16	2	18
V Ovariectomy	—	9	2	1	3
VI Tamoxifen	100	9	3	4	7

animals (Table 3) and caused the appearance of a persistent pattern of dioestrous vaginal smears (Nicholson, Walker & Maynard, 1980).

'Adjuvant' therapy of DMBA-induced mammary tumours

Clinical studies are currently underway with the antioestrogen, tamoxifen, and cytotoxic agents, given both alone and in combination, immediately after mastectomy. The hope is that such treatment, termed 'adjuvant' therapy, may increase the interval to recurrence of the disease and prolong survival time. Jordan (1978) has described a dosing regimen in the DMBA-induced tumour model which approximates to adjuvant therapy. In his model the drug is given,

Text-fig. 5. Development of new tumours in rats after withdrawal ICI 118630. (a) Growth patterns of new tumours at the end of treatment with 5 µg (○) or 0.5 µg (●) ICI 118630. (b) Response of tumours to ovariectomy.

Table 3. Effect of twice daily injections of 5 µg ICI 118630 for 20 days and ovariectomy on plasma oestradiol-17β concentrations

Treatment	Plasma oestradiol conc. (pg/ml)*
Control	33.6 (12.4–60.7)
ICI 118630	8.6 (3.4–15.0)
Ovariectomy	8.7 (3.5–24.0)

* Values are means, with ranges in parentheses, for 6 animals/group.

continuously or for a limited period, starting 30 days after tumour induction with the carcinogen, at a time when no tumours are palpable. Jordan (1978) has shown that in this situation tamoxifen is more effective than ovariectomy in inhibiting tumour development.

Thirty days after DMBA administration, treatment with ICI 118630 was initiated on a continuous basis in 20 rats with 40 animals serving as controls. The rats were palpated for tumours at weekly intervals and tumour sizes were recorded. Table 4 shows that the incidence of tumours was significantly reduced in animals treated with ICI 118630 (B. J. A. Furr, B. Valcaccia, C. Neal & F. G. Hutchinson, unpublished results).

Table 4. Effect of ICI 118630 on the appearance of DMBA-induced mammary tumours in rats

Treatment	No. of rats	Percentage of rats with tumours		
		28 days	42 days	70 days
Control	40	17.5	55.0	92.5
ICI 118630*	20	0	0	15.0

* Given continuously for 90 days, 30 days after the induction of tumours with DMBA.

Mechanism of action of ICI 118630 in causing tumour regression

Tumour regression induced by ICI 118630 could be due to a direct effect of the drug on the tumour, or to indirect effects through an action on the pituitary gland or ovary.

Frequent administration of pharmacological doses of LH-RH analogues causes refractoriness of the pituitary gland of rats (Sandow, Von Rechenberg, Jerzabek & Stoll, 1978),

Text-fig. 6. Effect of ICI 118630 on plasma LH concentrations. Rats were given ICI 118630 twice daily for 1 day (●) or 14 days (○) and killed by exsanguination at the times indicated. The arrows show the times of injection. Plasma LH values are the mean $\pm$ s.e.m. for 5 animals.

primates (Belchetz, Plant, Nakai, Keogh & Knobil, 1978) and man (Berquist, Nillius & Wide, 1979), and consequently prevents gonadal stimulation. However, the dosing regimen used in the dose-response study described here did not prevent LH release in the rat (Maynard & Nicholson, 1979). Text-figure 6 shows that, although reduced, sustained LH release still occurred in response to ICI 118630 after 14 days treatment; at this time tumour regression was clearly observed. It therefore seems unlikely that down-regulation of pituitary LH-RH receptors accounts for the antitumour activity. Alternatively, it is possible that the gonadotrophin released in response to ICI 118630 causes down-regulation of ovarian LH receptors which accounts for the observed reduction in plasma oestradiol-17 β . There is certainly ample evidence that there is a marked loss of LH receptors and the steroidogenic response to gonadotrophin stimulation after systemic administration of LH and hCG (Hsueh, Dufau & Catt, 1976, 1977; Sharpe, 1976; Auclair *et al.*, 1977). Moreover, a similar down-regulation of gonadal LH receptors is observed after administration of LH-RH analogues (Auclair *et al.*, 1977; Conti, Harwood, Dufau & Catt, 1977; Kledzik, Cusan, Auclair, Kelly & Labrie, 1978; Catt, Bakaul, Davies & Dufau, 1979).

Table 5. The effects of hCG and ICI 118630 on ovarian and uterine weights and plasma oestradiol concentrations in groups of 6 hypophysectomized rats

Treatment	Organ weight (mg/100 g body weight)		Plasma oestradiol (pg/ml)
	Ovaries	Uteri	
Vehicle	35.8 (32–39)**	55.4 (50–57)**	20.2 (8.5–50.5)*
hCG	71.9 (45–113)‡	152.0 (132–177)‡	50.1 (35.2–61.2)†
hCG + ICI 118630	39.3 (28–52)*	61.7 (45–90)*†	37.3 (21.3–47.2)*
ICI 118630	40.5 (31–45)*	58.8 (40–90)**	29.2 (16.9–34.4)**

Animals were treated twice daily for 3 days and once daily on Day 4 with vehicle, hCG (25 i.u./injection), ICI 118630 (5 μ g/injection) or a combination of hCG (25 i.u./injection) and ICI 118630 (5 μ g/injection). Animals were exsanguinated 1 h after the final injection on Day 4. The mean values are given and the range is shown in parentheses.

Significantly different from hCG-treated animals: * $P < 0.05$, ** $P < 0.005$ (Student's *t* test).

Significantly different from vehicle-treated animals: † $P < 0.05$; ‡ $P < 0.005$ (Student's *t* test).

Once again, however, it is unlikely that this represents the complete mechanism of ICI 118630 action since the concurrent administration of the drug with hCG to hypophysectomized rats completely abolishes the hCG-stimulated increases in ovarian and uterine weights (Table 5) and reduces circulating concentrations of oestradiol (K. J. Walker & R. I. Nicholson, unpublished). These events were observed in animals having no significant response to previously administered LH-RH and indicate a direct inhibitory action of ICI 118630 on the ovary with a subsequent reduction in circulating oestrogens. Such a concept is consistent with the findings of other workers using another synthetic LH-RH agonist, D-Leu⁶, des Gly¹⁰, Pro-ethylamide⁹-LH-RH (Rippel & Johnson, 1976), and is reinforced by the recent observations of LH-RH binding proteins in ovarian tissue (Clayton, Harwood & Catt, 1979; Reeves, Seguin, Lefebvre, Kelly & Labrie, 1980). Furthermore, Hsueh & Erikson (1979) and Hillier, Reichert & van Hall (1981) have shown that LH-RH analogues, including ICI 118630, will inhibit steroidogenesis by granulosa cells *in vitro* in response to gonadotrophin. Similarly, ICI 118630 reduces ovarian aromatase activity in intact rats (K. J. Walker & R. I. Nicholson, unpublished data).

Since DMBA-induced mammary tumours are responsive to prolactin (Sterental, Dominguez, Weissman & Pearson, 1963; Pearson, Llerena, Llerena, Molina & Butler, 1969; Bradley, Kledzik & Meites, 1976; Manni *et al.*, 1977), Danguy *et al.* (1977) concluded that the marked reduction in plasma prolactin found after treatment with D-Leu⁶, des Gly¹⁰, Pro-ethylamide⁹-LH-RH was the primary cause of tumour regression. A reduction in plasma prolactin was also caused by ICI 118630 treatment (K. J. Walker, R. I. Nicholson & B. J. A. Furr, unpublished data), as can be seen in Table 6. This was probably a consequence of the reduction in plasma oestradiol, because oestrogens regulate prolactin release in the rat (Meites, 1972; Jordan & Koerner, 1976). The interaction of oestrogen and prolactin in controlling growth of DMBA-induced tumours is worthy of further study before firm conclusions can be drawn about the relative importance of oestrogen and prolactin withdrawal on tumour growth.

Table 6. Effect of ICI 118630 on plasma prolactin concentrations in female rats

Time	Plasma prolactin (ng NIH rat prolactin RP-1/ml)
Pre-treatment	43.2 ± 9.6
After 5 days treatment	19.7 ± 8.6
After 10 days treatment	11.4 ± 2.9**
After 21 days treatment	10.2 ± 2.4**
1 day post-treatment	39.5 ± 13.3
2 days post-treatment	56.0 ± 23.3
3 days post-treatment	93.1 ± 30.7

Groups of 6 rats were given 5 µg ICI 118630 twice daily for 21 days. The rats were killed by exsanguination and the plasma prolactin concentrations were measured by radio-immunoassay. The values given are the means ± s.e.m.

** $P < 0.01$ compared with pre-treatment value; Student's *t* test.

Recent observations, on castrated and hypophysectomized rats, that LH-RH and D-Trp⁶-des Gly¹⁰, Pro-ethylamide⁹-LH-RH interfere with oestrogen and androgen action on the uterus and seminal vesicles respectively (Sundaram *et al.*, 1981), suggest that a direct action of the drug on the tumour is a possibility. However, we have been unable to alter the response to testosterone propionate in castrated immature rats with ICI 118630 or D-Trp⁶-des Gly¹⁰, Pro-ethylamide⁹-LH-RH (Table 7). Similarly neither analogue of LH-RH had an effect on uterine weight increases caused by oestradiol (Table 8). The reasons for the discrepancies between these results and those of Sundaram *et al.* (1981) are unclear. The principal difference between the studies

was that Sundaram *et al.* (1981) used hypophysectomized rats, although it is difficult, in our studies, to envisage a pituitary factor released by these analogues which would effectively prevent their direct inhibition of steroid action. Furthermore, in rats bearing DMBA-induced tumours the pituitary gland was present, making it unlikely that this mechanism is relevant to the antitumour properties of ICI 118630. In support of this conclusion are the findings of J. Yates & R. B. King (personal communication) that ICI 118630 has no effect on growth of the androgen-responsive, Shionogi mammary tumour line *in vitro*, both in the presence and absence of testosterone.

Table 7. Effect of D-Trp⁶, des Gly¹⁰, Pro-ethylamide⁹-LH-RH and ICI 118630 on ventral prostate weights in castrated immature rats given testosterone propionate (200 µg/kg) daily for 7 days

Experiment A		Experiment B	
Treatment	Wt of prostate gland (mg/100 g body wt)	Treatment	Wt of prostate gland (mg/100 g body wt)
Control	37.8 ± 2.8	Control	37.1 ± 1.7
D-Trp ⁶ , des Gly ¹⁰ , Pro-ethylamide ⁹ -LH-RH (10 µg/rat; ~100 µg/kg)	41.8 ± 2.0	Flutamide (2 mg/kg)	22.9 ± 1.1***
D-Trp ⁶ , des Gly ¹⁰ , Pro-ethylamide ⁹ -LH-RH (50 µg/rat; ~500 µg/kg)	37.3 ± 2.3	ICI 118630 (20 µg/kg)	39.8 ± 2.8
		ICI 118630 (100 µg/kg)	37.8 ± 2.6
		ICI 118630 (500 µg/kg)	38.1 ± 3.8

All rats were killed on Day 8. Values are mean ± s.e.m. for 5 rats/group. * $P < 0.001$ compared with control (Student's *t* test).

Table 8. Effect of ICI 118630 and D-Trp⁶, des Gly¹⁰, Pro-ethylamide⁹-LH-RH on uterine weights in 21-day-old female rats given 3 daily subcutaneous injections of 0.5 µg oestradiol benzoate alone or in combination with the LH-RH analogue; all rats were killed on Day 4

Experiment A		Experiment B	
Treatment	Uterine wt (mg/100 g body wt)	Treatment	Uterine wt (mg/100 g body wt)
Untreated control	53.0 ± 4.0	Untreated control	44.9 ± 1.6
Oestradiol benzoate	234.6 ± 12.1	Oestradiol benzoate	240.4 ± 5.5
Oestradiol benzoate + ICI 118630 (10 µg/rat)	241.1 ± 7.6	Oestradiol benzoate + D-Trp ⁶ , des Gly ¹⁰ , Pro-ethylamide ⁹ -LH-RH (10 µg/rat)	243.8 ± 10.2
Oestradiol benzoate + ICI 118630 (100 µg/rat)	246.1 ± 8.6	Oestradiol benzoate + D-Trp ⁶ , des Gly ¹⁰ , Pro-ethylamide ⁹ -LH-RH (100 µg/rat)	248.9 ± 6.8

Values are mean ± s.e.m. for 5 rats/group. The analogue doses of 10 and 100 µg/rat were equivalent to ~200 and ~2000 µg/kg.

It is concluded that withdrawal of oestrogen and prolactin in the DMBA-induced tumour model leads to tumour regression. This is caused initially either by a direct effect of the drug at the ovary, or by down-regulation of ovarian LH receptors induced by release of endogenous LH in response to the drug. Although there is no evidence that prolactin influences growth of breast tumours in man, oestrogens have been implicated in this respect. Thus treatment of pre- and peri-menopausal women with analogues of LH-RH may offer an alternative to oophorectomy as a therapy for advanced breast cancer and also as an adjuvant to surgery at mastectomy.

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