

Leuprolide acetate therapy in LH-dependent Cushing's syndrome: *in vivo* and *in vitro* observations

S.A. Bovenberg¹, G.F.F.M. Pieters^{1*}, L.J. Hofland², A.R.M.M. Hermus¹

¹Department of Endocrinology (531), University Medical Centre St Radboud, Nijmegen, the Netherlands, tel.: +31 (0)24-361 45 99, fax:+31 (0)24-361 88 09, e-mail: g.pieters@endo.umcn.nl, ²Department of Internal Medicine, Erasmus Medical Centre, Rotterdam, the Netherlands, *corresponding author

INTRODUCTION

In Cushing's syndrome, there is an excess of cortisol secretion due to hyperfunction of the adrenal cortex. Hypersecretion of cortisol may be ACTH dependent or independent. The latter is present in an adrenal adenoma/carcinoma or bilateral macronodular adrenal hyperplasia. In some of these patients cortisol secretion is driven by stimuli other than ACTH, such as gastric inhibitory polypeptide (GIP), vasopressin, catecholamines or luteinising hormone (LH).¹ LH-responsive Cushing's syndrome was first described by Lacroix *et al.*² In their patient *in vivo* investigations demonstrated that cortisol secretion was LH driven, but *in vitro* investigations were not performed.

Recently, *in vitro* studies in two patients with LH-responsive Cushing's syndrome demonstrated LH receptor mRNA in hyperplastic adrenal cells.³ Definitive proof that cortisol secretion was LH dependent could not be provided, because both patients refused LH-suppressive therapy. We now report another patient with LH-responsive Cushing's syndrome. Our patient responded to GnRH agonist therapy with a profound decrease in 24-hour urinary cortisol excretion, which proves that in this patient cortisol secretion was indeed LH driven. *In vitro* studies demonstrated LH receptor mRNA expression in the adrenal cells.

CASE REPORT

A 48-year-old woman presented with an 18-month history of fatigue, weight gain of 15 kg, muscular weakness and emotional instability. She reported an absence of menses for 2.5 years. She had had four uncomplicated pregnancies

with no signs of transient Cushing's syndrome and no abnormal weight gain. She was not taking any medication. Physical examination revealed a woman with classical clinical features of Cushing's syndrome (hypertension, moonface, buffalo hump, truncal obesity, atrophy of the skin and easy bruising). Endocrine evaluation demonstrated ACTH-independent hypercortisolism. A computed tomography showed bilateral macronodular adrenal hyperplasia. *In vivo* stimulation tests (*table 1*) showed increases in cortisol production after administration of GnRH (Etiapharma, Assen, the Netherlands, 100 µg iv), recombinant human LH (rhLH, Serono, Rome, Italy, 300 IU im), and cisapride (Janssen-Cilag, Tilburg, the Netherlands, 10 mg orally). No cortisol response was seen after administration of recombinant human FSH (rhFSH, Organon, Oss, the Netherlands, 300 IU im) and there were no abnormal cortisol responses to meals.

LH-responsive Cushing's syndrome associated with bilateral macronodular adrenal hyperplasia was diagnosed and treat-

Table 1

In vivo cortisol responses to GnRH, rhLH, cisapride and rhFSH (plasma cortisol values are expressed as µmol/l)

	BASELINE VALUE	PEAK VALUE
GnRH (100 µg iv)	0.54	0.77
RhLH (300 IU im)	0.23	1.30
Cisapride (10 mg po)	0.57	2.90
RhFSH (300 IU im)	0.48	0.55

ment was started with the GnRH agonist leuprolide acetate, 3.6 mg sc every four weeks. Serum LH levels declined rapidly from high postmenopausal values to lower than the detection limit of the assay after two months. This was accompanied with a decline in 24-hour urinary cortisol excretion, which became normal six months after starting treatment with leuprolide acetate (figure 1). However, the complaints and symptoms of Cushing's syndrome only partially disappeared and 24-hour urinary cortisol excretion was again above normal eight months after starting leuprolide acetate. Subsequently the patient underwent a laparoscopic bilateral adrenalectomy. Histological examination of the removed adrenal glands revealed macronodular adrenocortical hyperplasia. The size of the adrenals was 6 cm (right side) and 9 cm (left side). The results of the *in vitro* studies showed a significant increase in cortisol production after administration of ACTH₁₋₂₄ and metoclopramide (table 2). Remarkably, the response of cortisol to LH was absent *in vitro*. LH receptor mRNA expression was demonstrated in adrenal tissue although in low concentration (ratio of LH receptor mRNA to γ -actin mRNA was 0.68; this ratio was 0.58 and 1.07 in two control patients, respectively).

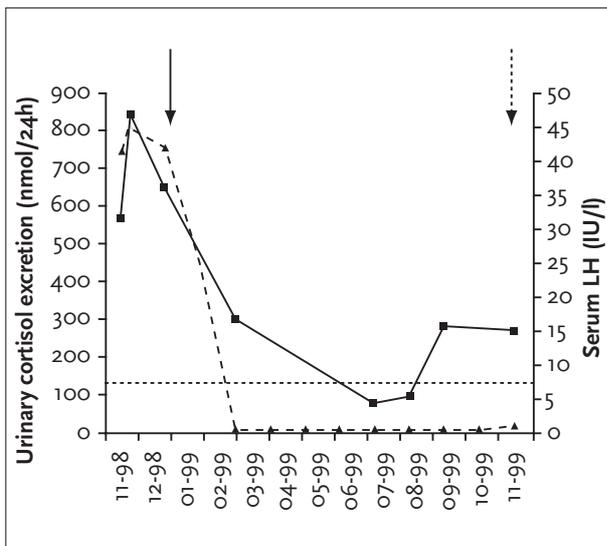


Figure 1
Urinary cortisol excretion (■) and serum LH levels (▲) before and during treatment with leuprolide acetate (3.6 mg sc every 4 weeks)

The first injection was given on 14 January 1999 (arrow) and the last one on 4 November 1999. Bilateral adrenalectomy was performed on 26 November 1999 (dashed arrow). The horizontal dashed line represents the upper limit of normal for 24-hour urinary cortisol excretion.

Table 2

In vitro cortisol responses of cultured adrenal adenoma cells to GnRH, rhLH, rhFSH, ACTH₁₋₂₄ and metoclopramide (two-hour stimulated cortisol values, expressed as the percentage of control, untreated cells)

TEST SUBSTANCE	CORTISOL VALUE (% CONTROL)	
Control		100 ± 8
GnRH	10 nM	92 ± 7
	100 nM	96 ± 1
RhLH	10 mIU/ml	99 ± 7
	50 mIU/ml	100 ± 10
	100 mIU/ml	95 ± 3
	1000 mIU/ml	100 ± 16
RhFSH	10 mIU/ml	87 ± 2
	50 mIU/ml	92 ± 10
	100 mIU/ml	90 ± 11
	1000 mIU/ml	101 ± 6
ACTH ₁₋₂₄	16 pg/ml	227 ± 4°
	32 pg/ml	267 ± 31°
	64 pg/ml	275 ± 32°
	128 pg/ml	310 ± 42°
	615 pg/ml	400 ± 30°
Metoclopramide	0.1 μM	184 ± 20°
	1 μM	372 ± 24°
	10 μM	454 ± 8°

Data are expressed as mean (n = 3) ± SD; °p < 0.01 vs control, °°p < 0.001 vs control. For methods see Feelders et al.³

DISCUSSION

In patients with ACTH-independent Cushing's syndrome due to an adrenal adenoma/carcinoma or bilateral macronodular adrenal hyperplasia, cortisol secretion can be stimulated by hormones other than ACTH due to aberrant expression of one or more G-protein-coupled receptors.¹ Until now such aberrant stimulation of cortisol has been demonstrated for GIP, vasopressin, 5 hydroxytryptamine (5HT₇ receptor), β-adrenergic receptor agonists and angiotensin II. Increased expression of eutopic receptors can also cause cortisol hypersecretion; this mechanism explains the stimulation of cortisol secretion in patients with ACTH-independent Cushing's syndrome by vasopressin, LH/human chorionic gonadotropin (HCG), 5 hydroxytryptamine (5HT₄ receptor) and leptin.¹

In our patient with LH-dependent Cushing's syndrome we demonstrated, in line with the data of Lacroix et al.,² that suppression of enhanced LH levels *in vivo* by leuprolide acetate led to suppression of cortisol levels. We can not explain the unexpected absence of a response of cortisol to LH *in vitro*. We hypothesise that stimulation of LH receptors needs, besides LH, a cofactor that is lacking *in vitro*. Furthermore, in our patient adrenalectomy was performed

after seven months of treatment with leuprolide acetate, which may have influenced the *in vitro* results, if suppression of LH levels causes downregulation of LH receptor expression. In our patient *in vitro* investigations demonstrated a cortisol response to ACTH and metoclopramide (that exerts its action via the 5HT₄ receptor), in line with the results of Feelders *et al.*³

Only very few patients with LH-responsive Cushing's syndrome and ACTH-independent macronodular adrenal hyperplasia have been described so far. In the first patient, described by Lacroix *et al.*,² administration of the long-acting gonadotropin-releasing hormone (GnRH) analogue leuprolide acetate led to suppression of endogenous LH and normalisation of cortisol production. In this patient cisapride and metoclopramide also stimulated plasma cortisol. Feelders *et al.*³ described two women with LH-responsive Cushing's syndrome and ACTH-independent macronodular adrenal hyperplasia. Both patients had aberrant responses to GnRH, LH, hCG, cisapride and metoclopramide. mRNA encoding the LH receptor was slightly higher in the macronodular adrenals from these patients than in normal adrenals.

In our patient with ACTH-independent macronodular adrenal hyperplasia and Cushing's syndrome with aberrant responses to GnRH, LH and cisapride *in vivo* and to metoclopramide *in vitro* suppression of endogenous LH with leuprolide acetate only partially improved hypercortisolism. This may be due to persistent aberrant stimulation of cortisol secretion via 5HT₄ receptors, but may also indicate

the presence of either other, unidentified, aberrant receptors or another mechanism that regulates cortisol secretion. Many questions with respect to Cushing's syndrome variants secondary to aberrant hormone receptors remain unanswered. Nevertheless, the phenomenon of cortisol stimulation via receptors other than the ACTH receptor opens new possibilities to treatment of ACTH-independent Cushing's syndrome.

ACKNOWLEDGEMENTS

Dr A.P.N. Themmen, Erasmus Medical Centre, Rotterdam, the Netherlands, is gratefully acknowledged for the LH receptor mRNA analyses.

REFERENCES

1. Lacroix A, Baldacchino V, Bourdeau L, Hamet P, Tremblay J. Cushing's syndrome variants secondary to aberrant hormone receptors. *Trends Endocrinol Metab* 2004;15:375-82.
2. Lacroix A, Hamet P, Boutin JM. Leuprolide acetate therapy in luteinizing hormone-dependent Cushing's syndrome. *N Engl J Med* 1999;341:1577-81.
3. Feelders RA, Lamberts SWJ, Hofland LJ, et al. Luteinizing hormone (LH)-responsive Cushing's syndrome: the demonstration of LH receptor messenger ribonucleic acid in hyperplastic adrenal cells, which respond to chorionic gonadotropin and serotonin agonists *in vitro*. *J Clin Endocrinol Metab* 2003;88:230-7.